Assessment of pharmaceutical residues in industrial wastewaters

MSc. Research Thesis by

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Declaration

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Abbreviations

ACS	American Chemistry Society
AOP	Advanced Oxidative Process
API	Active Pharmaceutical Ingredient
AC	Activated Carbon
AS	Activated Sludge
BFR	Biofilm Reactors
CFM	Crude Famotidine
COD	Chemical Oxygen Demand
DMF	N,N,-Dimethylformamide
EPA	Environmental Protection Agency
ESI	Electrospray Ionisation
FDA	Food and Drug Administration
GCI	Green Chemistry Institute
GMP	Good Manufacturing Process
HPLC	High Pressure Liquid Chromatography
HCl	Hydrogen Chloride
IPPC	Integrated Pollution Prevention Control
K_2CO_3	Potassium Carbonate
KCl	Potassium Chloride
LC-MS	Liquid Chromatography Mass Spectrometry
MeOH	Methanol
OEE	Office of Environmental Enforcement
OOSPAR	Oslo Paris Convention
PAT	Process Analytical Technologies
PFM	Pure Famotidine
PFP	Pentaflourophenyl Propyl
PPCP	Pharmaceuticals and Personal Care Products
QC	Quality Control
RSD	Residual Standard Deviation
SBR	Sequencing Batch Reactors

SPE	Solid Phase Extraction
SPFM	Semi-Pure Famotidine
TEA	Triethylamine
TPN	3-(2-Guanidino-thiazol-4-yl-methylthio)-propionitrile
UABP	Upflow Anaerobic Biofilter Process
WFD	Water Framework Directive
WWA	Wastewater Tank (Mixed Solvent)
WWC1	Wastewater Tank (Aqueous)
WWTP	Wastewater Treatment Plant

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Abstract

Active pharmaceutical ingredients (APIs) are entering the environment through various pathways and emissions of effluents from pharmaceutical production plants are one such source. The production process of a pharmaceutical for the treatment of stomach ulcers manufactured at a pharmaceutical production plant in Ireland was studied. Data detailing mass flow quantities and compositions were compiled. This occurred over a 6 week period following a two week plant shutdown. A computer software programme, SuperPro Designer v 5.0, was used to estimate the efficiency of the production process, mass flows in waste streams and process streams. Several assumptions were made in modelling the actual process including the percentage purity of the raw material, the percentage intermediate formation, the percentage product formation and the percentage losses during product purification. In order to compare predicted and actual concentrations, an LC-ESI-MS/MS method was developed to detect the raw material and product in wastewater. A sample point where water from the process collects and a sample point prior to the wastewater treatment were used. Concentrations in the mg/L range were detected. Mass balances of process streams in the pharmaceutical production facility were used to estimate the quantities of the raw material and product lost to the waste streams which were then compared with the model created using SuperPro Designer v5.0. The model was useful in predicting losses of both raw material and product and actual wastewater analysis confirms this. Sampling points at each centrifuge in the plant would allow the losses to be more accurately quantified.

Presentations

Oral presentation

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Chapter 1

Introduction

1.1 Presence of pharmaceuticals in the environment

It is well documented that there are detectable quantities of pollutants, including pharmaceuticals in the aquatic environment (Glassmeyer et al., 2009, Heberer, 2002, Hirsh et al., 1999). Pharmaceutical and Personal Care Products (PPCP) are contaminants in the environment which have traditionally not been monitored (Aga, 2008). They have the potential for adverse health effects, especially endocrine disrupting compounds (Bolong et al., 2009). There has been a global detection of pharmaceuticals in environmental samples as a result of improved analytical capabilities and detailed field surveys (Focazio et al., 2008, Webb, 2003, and Daughton, 2001,). Methods of detection for these micro pollutants have improved with the advent of liquid chromatography coupled with tandem mass spectrometry and there has been a significant increase in reporting the presence of PPCPs in the environment in the literature (Aga, 2008).

The high polarity and low volatility of most pharmaceuticals means that they are likely to remain in the aquatic environment (Van der Voet, et al., 2004). Six of the main environmental journals have witnessed a six fold increase in publications regarding the fate of pharmaceuticals in the environment (Aga, 2008). Many pharmaceuticals are unlikely to be a risk to the aquatic environment because of low concentrations combined with low toxicity but other pharmaceuticals such as natural and synthetic sex hormones have been shown to pose considerable risks (Bolong et al., 2009). New pharmaceuticals may be more persistent in the environment as they are designed to withstand degradation.

1.2 Entry to the environment

APIs enter the environment via a variety of pathways, including discharge of raw and treated sewage. This occurs by flushing unwanted pharmaceuticals

down the toilet/sink or by the presence of unmetabolised compounds excreted in faeces and urine (Daughton and Ruhoy 2008). The quantity of publications on the fate of APIs in the environment - such as sorption and mobility in soil (Lucas and Jones 2009), removal through tertiary treatments (Muñoz et al., 2009) (Klavarioti, et al., 2009), biodegradation (Kümmerer et al., 2000), and photodegradation (Tixier et al., 2003) - indicates the importance of research in this field. Secondary treatment of wastewaters is generally ineffective at degrading pharmaceuticals (Klavarioti et al., 2009).

The reduction of pharmaceuticals entering the environment may be a more important and more effective strategy of removal than attempting to eliminate them once in the environment (Ruhoy and Daughton, 2008). Some pharmaceuticals are persistent even after wastewater treatment, such as gemfibrozil and carbamazepine (Lacey et al., 2008). The pharmaceutical industry is both directly and indirectly responsible for the presence of these compounds and little has been done to reduce the quantity of pharmaceuticals released (Khetan and Collins, 2007). Although pharmaceuticals originate at manufacturing plants, little attention has been given to their wastewater effluents (Klavarioti et al., 2009). With advances in medical technology and growing healthcare spending, the consumption and usage of pharmaceuticals is expected to expand as new drugs enter the market and thereby increasing pharmaceutical loading on the environment.

There are several reasons for a pharmaceutical plant to reduce the quantities of APIs in effluent, including: (i) reduction of the environmental impact, (ii) improved public perception of the industry and (iii) to avoid large fines imposed by regulatory bodies. The recovery of high value products should be part of the production process or at the latest, the purification step. Recovery of product should not occur after purification or polishing steps. Legislation is a key driver in the reduction of pollution from the pharmaceutical industry and it is becoming more stringent as analytical techniques improve (Bolong et al., 2009). Legislation regarding water quality in the United States, Europe and

specifically Ireland is discussed, with the European Water Framework Directive (WFD) owing to the tightest regulation.

Recovering APIs from process streams with more efficiency will mean that less APIs will be present in the waste streams. Methods of recovery for an API are dependent on several factors including molecular weight, compound classification (e.g. protein, small molecule, antibiotic etc.) and cost. Chromatography and membrane technology are the main separation techniques employed in the pharmaceutical industry (Bolong et al., 2009, Van den Heuvel, 2009, Sofer, 1995). For the small molecule pharmaceutical industry, downstream processing usually entails filtration technology to remove impurities followed by crystallisation steps.

1.3 Treatment Options

Several technologies are available to degrade pharmaceutical residues in the municipal wastewater area, including conventional Activated Sludge (AS) plants, Activated Carbon (AC), (Watkinson et al., 2007), Biofilm Reactors (BFRs), Sequencing Batch Reactors (SBRs) (Mohan et al., 2006), Membrane Bioreactors (MBRs) (Radjenović et al., 2009) and Upflow Anaerobic Biofilter Processes (UABP)(Chen et al., 1994). These technologies have been shown to remove pharmaceuticals of certain classes more efficiently than others. Advanced oxidation processes for the removal of pharmaceuticals, though effective, are expected to be an expensive endeavour for municipal wastewater. As initial concentrations of APIs are very low the treatment cost per unit mass may be excessive and therefore AOPs are more suited to industrial effluents (Klavarioti et al., 2009). The long-term impact of low concentrations of APIs on both the environment and human health is still unknown (Crane et al., 2006).

Due to the high concentration of pollutants in industrial effluents, recovery of solvents, products and raw materials may be of more benefit than treatment,

as it increases the efficiency of the process. Municipal wastewaters have been shown to have pharmaceuticals at concentrations of ng/L, whereas the effluents of some hospitals and pharmaceutical plants are much higher, in the mg/L range (Klavarioti et al., 2009). In most cases of pharmaceutical effluent, specific quantities of pharmaceuticals are either not monitored or are not publicised. Chemical Oxygen Demand (COD) of streams are reported for pharmaceutical industrial effluent and can be in the region of 670-2700mg/L (Klavarioti, 2009, Xing et al,. 2006, Hofl et al., 1997). Introduction of regulations to reduce the entry of API's to the environment via production plants is the only feasible option for rapid development of technology (Linninger et al., 2001). There has been a lack of economic incentives to develop "waste-free" processes in the pharmaceutical manufacturing industry (Garcia et al., 2004) and practices aimed at water usage reduction were rarely employed (Garcia et al., 2008). The potential risks associated with releases of pharmaceuticals into the environment have become an increasingly important issue for environmental regulators and the pharmaceutical industry (Crane, et al., 2006).

1.4 Legislation

Chemical synthesis has traditionally been at the core of pharmaceutical production. Improvements in pharmaceutical production facilities have come about due to economic incentives and tighter regulations. Legislation has had a major impact on the composition of effluent from pharmaceutical facilities as demonstrated in the Astellas 2008 Annual Environmental Report for the EPA. Improvements in purification technology have undoubtedly been attributed to demands from both customers of APIs and regulatory bodies (Févotte, 2007).

The potential risks associated with the release of pharmaceuticals into the environment have become an increasingly important issue for environmental regulators and the pharmaceutical industry (Crane et al., 2006). Little has been done to reduce the quantity of pharmaceuticals released to the environment (Khetan and Collins, 2007). Only in 2007, in the United States, the first federal recommendations for proper disposal of expired or unused pharmaceuticals

were introduced. While discouraging flushing of pharmaceuticals, it recommended using State and local collection programs or disposing to rubbish bins. The latter disposal method is only to be taken when no collection program is available. Prior to this, it had been recommended to dispose of drugs by flushing down the toilet (Glassmeyer, et al., 2009).

A lack of awareness regarding contamination of the environment by pharmaceuticals has been highlighted in a survey of residents in Southern California. Less than half of respondents were aware that pharmaceuticals compounds were present in treated wastewater (Kotchen, et al., 2009). Nearly half (49%) used a rubbish bin to dispose of unused pharmaceuticals and 28% used a toilet/sink, whereas 10.6% returned the unused drugs to a pharmacy or hazardous waste centre. A survey conducted in the United Kingdom reported a similar disposal rate to landfill, but only 11% said they flushed them down the toilet with 21.8% returning to the pharmacy (Bound and Voulvoulis, 2005). Minimising the disposal pathway of pharmaceuticals could be more effective and less costly than extensive Wastewater Treatment Plant (WWTP) retrofitting.

In Ireland, a pharmacist “may accept the return of a medicinal product” (S.I. No. 488 of 2008), but no regulations regarding the disposal by consumers have yet been made. Similarly, in the United Kingdom discarded pharmaceuticals are defined as clinical waste and are controlled by the Special Waste Regulations 1996 (HMSO, 1996). This legislation requires the pharmaceuticals to be disposed of in designated hazardous waste landfill sites or to be incinerated. However, once obtained by a member of the public, these types of waste are regarded as household waste and are not subject to any controls (Bound and Voulvoulis, 2005). New pharmaceuticals designed to withstand degradation and with more specific biological targets, may become more persistent in the environment. It is suggested that pharmaceutical producers should highlight environmental precaution when designing new drugs (Gunnarsson and Wennmalm, 2008).

In the United States, the Clean Water Act (1977) was brought into law in order to restore and improve the quality of all water sources. The aim was to eliminate the discharge of pollutants to navigable waters by 1985. To achieve this, federal funding was committed to construct publicly owned wastewater treatment works to develop technology which could eliminate pollutants before entering surface waters (Federal Water Pollution Control Act, 1972). No references to pharmaceuticals are made in this legislation. The paucity of regulation regarding pharmaceuticals at the time, compared with today, is indicative of the advances made by the regulatory authorities. The Oslo Convention was commissioned in 1972 to protect the marine environment of the North-East Atlantic. The Paris Convention of 1974 broadened this scope to cover land-based sources and off-shore industry. This was up-dated and extended resulting in a new annex, the 1992 OSPAR Convention (www.ospar.org). In 1989, the Irish Environmental Protection Agency carried out the first systematic nationwide assessment of drinking water quality. A total of fifty three bacteriological, chemical and physical parameters were examined (Flanagan, 1991). The quality of drinking water was generally good, with private group schemes showing breaches in microbiological contamination. This is reflected in a subsequent report (Clabby et al., 2008).

A less-investigated path of entry of pharmaceuticals to the environment is from the production processes. Diminution of released APIs in waste streams may be encouraged by a change in the regulatory environment (García et al, 2008). Residues of pharmaceuticals in aquatic systems are not yet included in regular monitoring programs. This is attributed to the high cost of equipment (Buchberger, 2007). The persistence and occurrence of endocrine disrupting compounds is attributed to the "nonexistence of limiting regulations, especially for new compounds, by-products, pharmaceuticals and PPCPs as related to the water and wastewater treatment industry" (Bolong et al., 2009). The European Water Framework Directive (WFD) set up objectives to achieve "good water status" for all European waters by 2015. In the WFD, a clear structure has been

set out to enable these objectives (Loos, et al., 2008). Not all Irish water meets this “good status” (Clabby et al., 2008). Nitrogen and phosphorous are the primary pollutants and enter surface waters from agriculture, sewage and detergents, amongst others. In the WFD, no specific regulations regarding pharmaceutical contamination of either industrial or municipal wastewaters are set out. However, the WFD includes 33 priority chemicals and 8 pollutants that will be subject to cessation or phasing out over the next 6 years (Official Journal L 327/22, 2000). The production of a number of these has been prohibited in a number of countries, including Ireland. Separate to that, the European Reach legislation (Official Journal L 396/1, 2006) seeks to provide a legal framework for dealing with chemicals ensuring a high level of health and environmental protection (Hogenboom, et al., 2009). The objective of the Reach legislation is the classification of chemicals and compilation of data such as environmental fate, physical and chemical properties and physicochemical properties, toxicological data, compositional data, chemical identity, volume of production, uses and exposure data (Official Journal L 396/1, 2006). Even though Astellas products are currently not on the priority list, it is possible that they may be included in time to come.

According to the Food and Drug Administration (FDA), the Good Manufacturing Practice (GMP) guidance for manufacturing and processing of APIs requires material accountability and traceability, as well as mass-balancing of all reactions during manufacturing. Process analytical technologies (PAT) were introduced in late 2002 by the FDA, to allow the introduction of new technologies which analyse and control manufacturing during processing. The analysis of raw and in-process materials may reduce risks to quality and regulatory concerns while improving efficiency of the process. This may also reduce the quantity of pharmaceuticals entering the environment. In pharmaceutical plants, the actual yields are compared with expected yields at designated steps in the production process. Expected yields with appropriate ranges are established and deviations from critical process steps should be

investigated (FDA, 2001). These measures are gradually making pharmaceutical manufacturers aware of their environmental impact.

Integrated pollution prevention control licences (IPPC) are required by industries which discharge pollution caused by certain substances into the aquatic community (Official Journal L 24/8, 2008). European law requires enforcement of these Directives. IPPC licences require production facilities to review the way in which they conduct their business, to innovate where necessary and to decouple production from environmental pollution. The Office of Environmental Enforcement (OEE) enforces these regulations. In the United States, no maximum limit of PPCPs in either drinking or natural waters has been regulated. However, when environmental concentrations of pharmaceuticals exceed 1µg/L, the Food and Drug Administration does require ecological testing and evaluation of pharmaceuticals (Bolong, et al., 2009)

1.5 Link between downstream processing and legislation

Legislative efforts to reduce the environmental impact of pharmaceutical companies have shifted the mindset of the industry to adopt greener technologies. In 2005, the American Chemical Society (ACS), Green Chemistry Institute (GCI) and other leading global pharmaceutical corporations developed the ACS GCI Pharmaceutical Roundtable to encourage the use of green chemistry in drug discovery and production of active pharmaceutical ingredients (Constable et al., 2007). Solvent-less cleaning and replacement of dipolar aprotic solvents were discussed, amongst others. This type of production may very well reduce the quantities of pollutants entering the environment, but for validated methods, this may not be feasible. Therefore, other means of pollution prevention are necessary. Pharmaceutical waste streams typically have high COD concentrations, compared with municipal waste water (Klavarioti et al., 2009). There is a general paucity of literature concerned with recovery of pharmaceutical products from industrial wastewater, most of which deal with the recovery of proteins by means of

membrane technology (Oatley et al., 2005). One would speculate the reason for the lack of pharmaceutical recovery is propriety or that there is currently very little research being carried out in this area, or both. Whichever the case may be, there is sufficient evidence from other sectors that technologies exist for the recovery of pharmaceutical from wastewater, possibly by membrane technology (He et al., 2004). There are several publications on recovery of waste by-products from wastewaters, including heavy metals from the wastewater of the electrical industry (Cui and Zhang, 2008) and dyes from the textile industry (Muthuraman et al., 2009, Mittal et al., 2006). These technologies may be applied to the pharmaceutical industry, in conjunction with wastewater treatment (as mentioned in section 1.3), to ameliorate the quality of water in effluents of plants.

1.6 Modelling

The operation of pharmaceutical plants must be understood in order to predict the emission points of pharmaceutical contaminants. One needs to apply the conservation of mass when searching for pollutants coming from pharmaceutical plants. Mass balancing is a fundamental step involved in theoretical analysis. To understand the performance of a system, two methods of analysis are possible: empirical investigation and mathematical modelling. The former would require several experiments to be performed. This may not provide sufficient information, as correlations to cover every process eventuality are necessary to do this (Ingham et al., 1994). There are several categories of models but they can generally be separated into two types: steady-state and dynamic models (Tirronen and Salmi 2003). For steady-state models, the rate of change of mass is zero and therefore there is no accumulation in the system (Ingham et al., 1994). Continuous production processes are steady-state models, whereas batch and semi-batch systems are dynamic models, as the rate of change is a non-zero value (Harrison et al., 2003). The level of detail in any model depends on its purpose – a basic model is produced and layers of complexity are added until the model meets its

requirements (Gosling, 2005). Mathematical modelling attempts to describe both actual and probable behaviour in a process (Dunn et al., 2000).

Pharmaceutical plants are usually complex dynamic systems designed to optimally perform at minimum cost. The traditional sequential procedure followed to design pharmaceutical plants involves the development of a flexibility analysis, commonly based on steady-state calculations and knowledge gained from similar production processes (Ricardez Sandoval et al., 2008). The use of dynamic models, as opposed to steady-state models for pharmaceutical plant analysis, has only recently been made possible through the use of powerful computer simulation software (Ingham et al., 2007). Mathematical models can be used to simulate, analyse and optimise the processes involved in chemical and pharmaceutical production (Tan et al., 2004). Optimisation includes direct maximisation of product yields while increasing efficiency of the process. The term also accounts for the prediction of API loss and the facilitation of their recovery from waste streams (Bowen and Wellfoot, 2002).

Models can be used to identify where and when measurable concentrations of pharmaceuticals will occur in the environment even when the actual concentrations are in the ng/L range and are often associated with complex matrices (sediment, soil, etc.) (Jørgensen and Halling-Sørensen, 2000). Models can also be used, for example, to predict the degradation of pharmaceuticals in waste treatment processes (Seth et al., 2007). Comparison of predictions with actual measurement can serve to highlight inadequacies of the models and lead to their refinement. Models may start from simple mass balances and can be progressively refined.

The purpose of creating a model is to simulate, as accurately as possible, what is happening in a system. Chemical and pharmaceutical companies use a range of software tools to analyse complete processes. Computer programs use several mass balance equations and allow them to be solved rapidly. These

tools allow the generation of process flow diagrams, mass and energy balancing as well as estimation of operating costs.

The modeller must identify important variables and their effect on the system. Understanding critical parameters and generating mathematical equations gives the modeller further insight to the system. Once the model has been formulated, it can be solved and then compared with experimental data. Deviations from actual data may be used to further redefine or refine the model until good agreement between it and experimental data is achieved (Dunn et al., 2000). It is important to calibrate and validate the applied models against real data.

1.7 Research overview

Pharmaceuticals which have not been completely removed by wastewater treatment have been found to be present in surface waters (Cooper et al., 2008, Ternes, 1998). Famotidine - a pharmaceutical produced by Astellas Ltd., Mulhuddart, Co. Dublin - is indicated for active and maintenance therapy of various types of ulcers and hypersecretory conditions (Fahmy and Kassem, 2008). Famotidine is a histamine H_2-receptor antagonist for treatment of ulcers in the stomach and intestine, its molecular structure is presented in Figure 1.1 (Helali et al., 2008). Its mechanism of action selectively antagonises histamine H_2 receptors inhibiting stomach acid production.

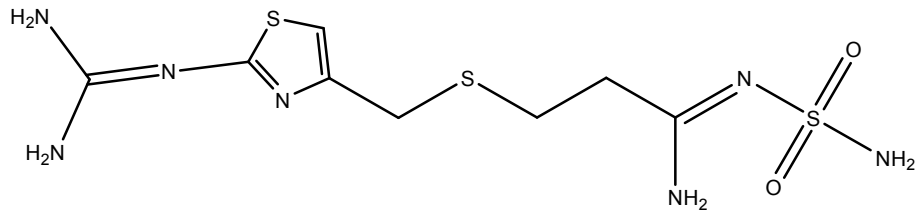

Figure 1.1 Famotidine structure.

Famotidine's pharmacological effects, site of action, and clinical uses are the same as for the other H_2-receptor antagonists, but on equimolar bases,

famotidine is reported to be about 7.5 and 20 times more potent than ranitidine and cimetidine, respectively (Fahmy and Kassem, 2008). Famotidine's potency is of concern when one considers the presence of pharmaceuticals in surface waters and their impact on the aquatic organisms (Daughton and Ruhoy, 2008). Little information is known about the raw material TPN (see Figure 1.2). The pharmaceutical industry is becoming more cognisant of its impact on the environment and has begun to take preventative action of pollution reduction. Astellas has collaborated with DCU to assess the wastewater on site with a view to identifying further means for improving the production process efficiency and reducing their environmental impact. One way to achieve this goal is to model a production plant's chemical processes and conduct mass balances which may show where product is unaccounted for and highlight stages in these processes which can be optimised to reduce these losses. SuperPro Designer v5.1®, a software package that specialises in modelling chemical unit operations and scheduling conflicts, is widely used within this industry and was chosen to model the production of famotidine. The parameters which can be modelled using SuperPro Designer include mass transfer, energy usage, plant economics and employee costs. In the development phase of a SuperPro Designer model, the process for the selected chemical route is laid out on a flow sheet. Mass balances, preliminary energy balances and basic recipes are generated for the process. The physical properties for pure compounds and mixtures are acquired from literature and data banks or they are estimated with appropriate physical property data. The scheduling of the unit operations are set out and gantt charts may be generated to characterise process bottlenecks. For the scope of this research, energy balancing and plant economics were omitted and only mass transfer was examined.

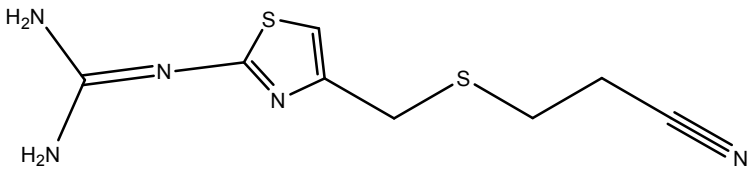

Figure 1.2 TPN Structure.

Two polymorphs of famotidine are produced at the plant at Astellas Ltd. These are A-form and B-form crystals. For HPLC methods of detection, A-form polymorph was supplied by Astellas. Scanning Electron Microscope images of TPN and famotidine are seen in Figure 1.3.

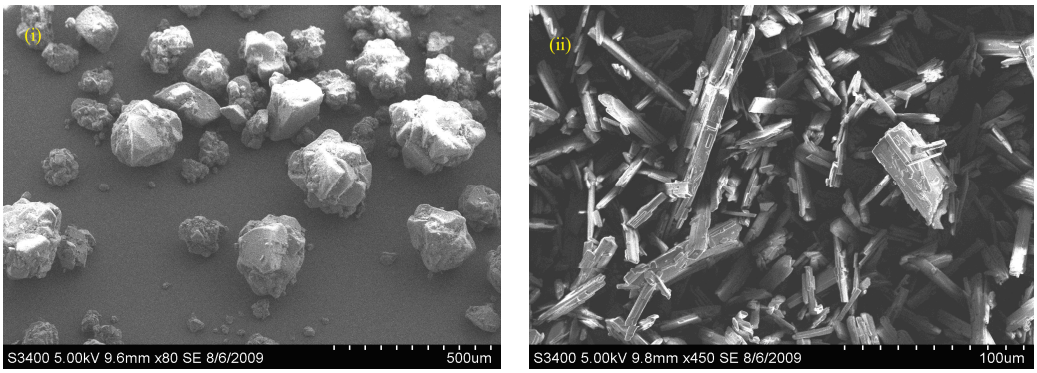

Figure 1.3 scanning electron microscope images of (i) TPN particles and (ii) A-form famotidine molecules.

During the purification process, both polymorphs are used at different stages to seed dissolved famotidine solutions and crystallise the product. The filter mesh-sizes in the basket centrifuges are different to increase the purification process efficiency. For this reason two types of crystal are used. In the final purification stage the production process splits to either the A-form route or the B-form route. The polymorphs are different sized crystals and are sold to two separate markets. A schematic of the production process is shown in Figure 1.4.

The objective of the project was to model the production of famotidine using SuperPro Designer in order to predict where losses of raw material and product may occur. This was to be then corroborated using experimental analysis of real process wastewaters from Astellas.

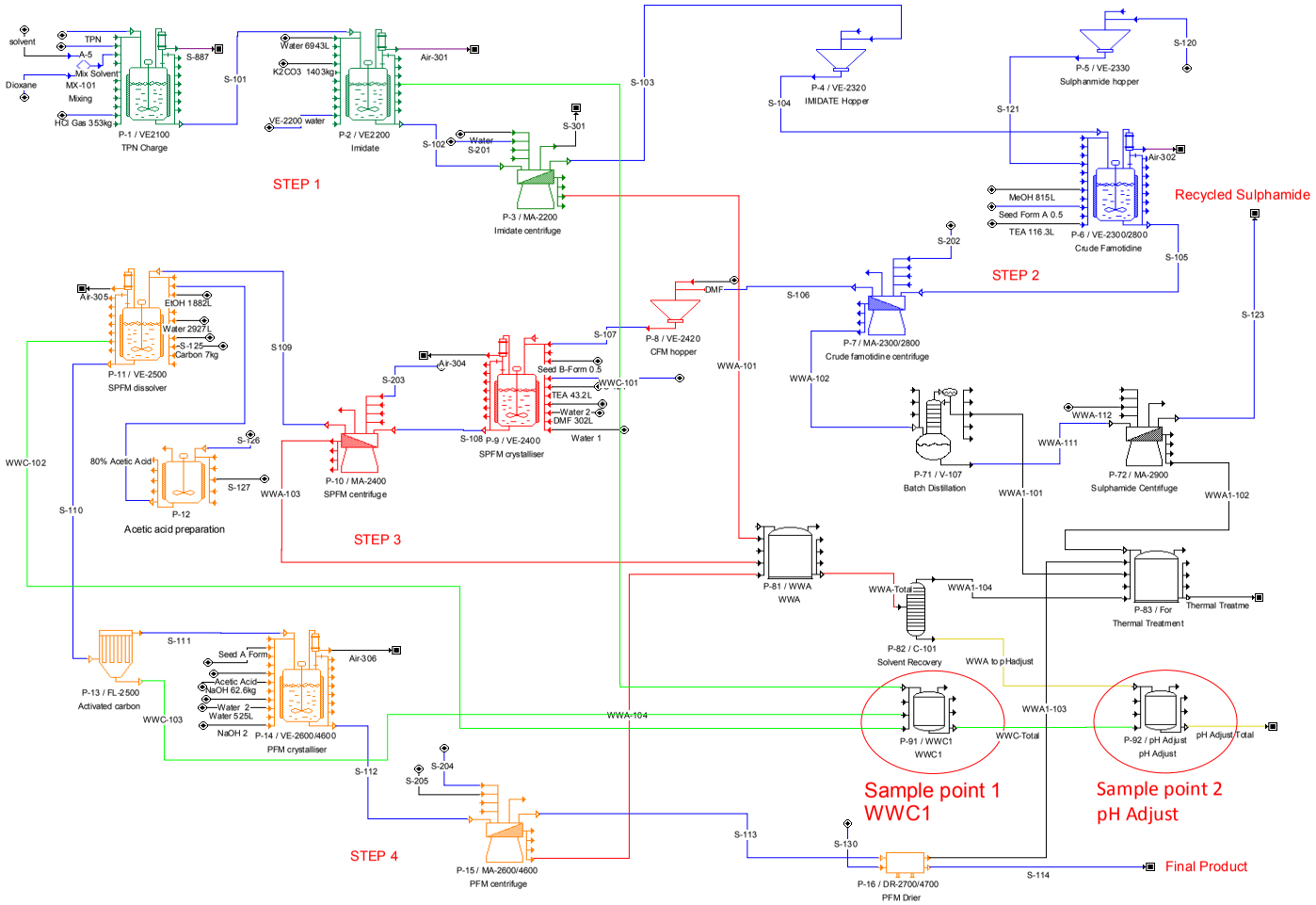

Figure 1.4 SuperPro Designer v5.0 flow diagram of famotidine production process (blue streams) and wastewater washes (green, red, yellow and black streams). Both sample points are circled.

Chapter 2

Materials and methods

2.1 Materials

Methanol and water were purchased from Fisher Scientific Ltd., Dublin, Ireland and were of LC-MS grade. Phosphoric acid solution (85%) and hydrochloric acid solution (≥37%), along with dichlorodimethylsilane and toluene, both HPLC grade, were purchased from Sigma-Aldrich, Dublin, Ireland. Formic acid (≥98%) and ammonium hydroxide solution (25%) were purchased from Fluka, Buchs, Switzerland. The analytes for investigation were famotidine (3-(((2-((aminoiminomethyl)amino)-4-thiazolyl)methyl)thio) -N- (aminosulfonyl) propanimidamide) (≥99%) and TPN (3-(2-Guanidino-thiazol-4-yl-methylthio)-propionitrile) (≥99%) and were obtained from Astellas Pharma Co. Ltd., Dublin, Ireland. A reverse phase Luna-pentaflourophenyl propyl (PFP) column 3.5µm particle, 150 x 4.6mm was used for standard HPLC analysis and a Luna PFP 3.5µm particle, 150 x 2.1mm was used for LC-MS analysis and were purchased from Phenomenex Inc., United Kingdom. Strata-X-C (3ml/200mg) solid phase extraction cartridges were also purchased from Phenomenex Inc., United Kingdom.

1000mg/L stock solutions of each analyte were prepared in methanol and stored at 4°C. Working standards were prepared by diluting these stock solutions using mobile phase.

HPLC vials (APEX Scientific, Co. Kildare, Ireland) and centrifuge vials (Fisher Scientific Ltd.) were made of amber glass to prevent degradation of analytes by light. All solvents used in HPLC analysis were filtered through Pall nylon filters (0.2µm pore size, 47mm diameter) and degassed by sonication for 30 min prior to use. Whatmann no 3. glass-fibre filters were used for sample filtration. SuperPro Designer V 5.1® (Intelligen, Boston, MA, USA) was used to model the production process of famotidine.

2.2 Glassware preparation

All glassware used was silanised by rinsing thoroughly with a 10% (v/v) solution of dichlorodimethylsilane in toluene followed by two toluene rinses and then two methanol rinses. This was to prevent any pharmaceutical residue adsorbing to the glassware.

2.3 Method Development

Famotidine and TPN were expected to be present in wastewater at the Astellas production plant. A quantifiable method of detection for famotidine and TPN was developed to validate the model. High performance liquid chromatography (HPLC) was used to detect both analytes. This method was then transferred to a liquid chromatography mass spectrometer (LC-MS/MS) to measure the analytes quantitatively and qualitatively. Other compounds which elute from the HPLC column at the same time as famotidine and TPN were present in the wastewater as other peaks were observed in chromatograms. The mass spectrometer first positively ionises famotidine and TPN. The ions are isolated in an ion trap and are fragmented to their respective daughter ions (see Figure 2.2). Famotidine is always fragmented to an ion of 259m/z and TPN to 155m/z. Therefore LC-MS/MS is a confirmation step as well as a quantitative method. A solid phase extraction (SPE) method was developed for both analytes but the concentration of famotidine in actual wastewater was quantifiable without pre-concentration.

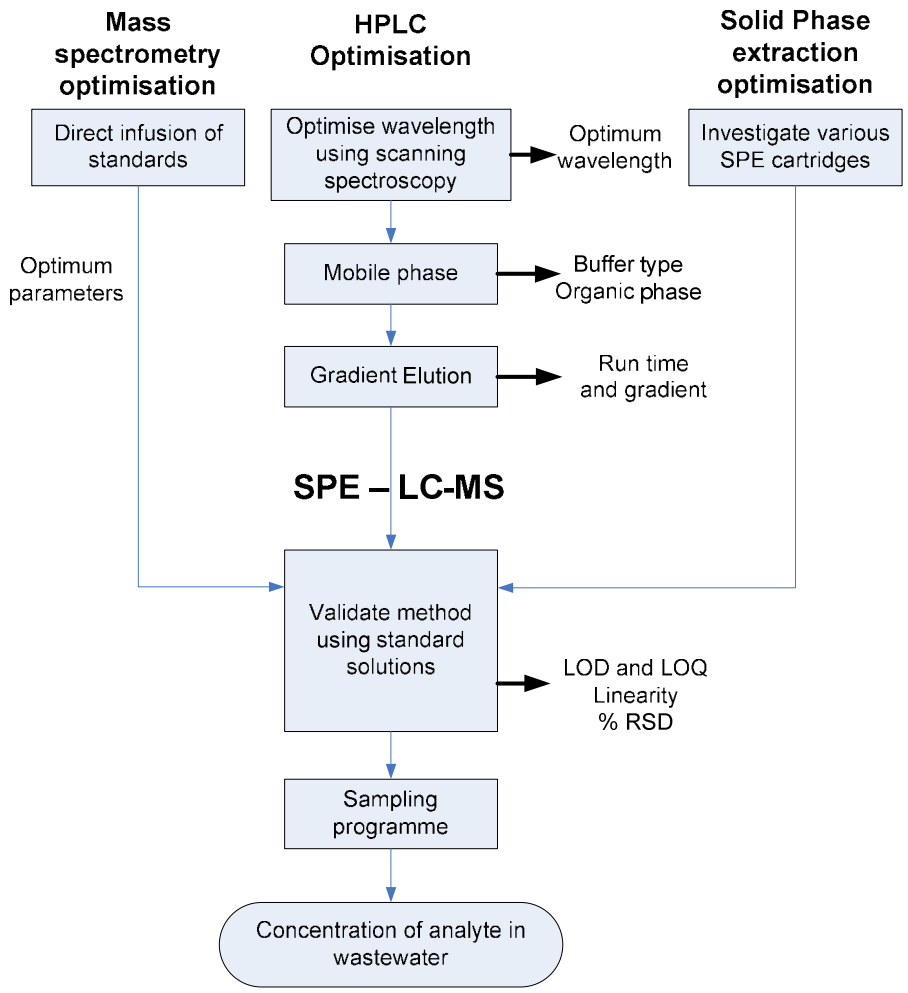

Figure 2.1 Method development flow chart.

An Agilent 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) with a UV-Vis detector was used for the development of the HPLC method. Separation of the analytes was performed with a 3.5µm particle, 150 x 4.6mm, Luna PFP reverse phase column (Phenomenex, UK). Varying ratios of methanol and water with formic acid (pH 2.7) and acetonitrile and water with formic acid (pH 2.9) allowed the identification of the optimum mobile phase for separation of both analytes. It was determined that a mobile phase composition of 23% methanol / water with 0.1% formic acid (pH 2.7) was the optimum. After running the sample, a gradient mobile phase with 90% methanol/water v/v with 0.1% formic acid was used to remove unwanted organic contaminants from the column which may be present in the wastewater (see Table 2.1).

Table 2.1 HPLC gradient timetable. A: 23:77 v/v (methanol/water) with 0.1% formic acid. B: 90:10 v/v (methanol/water) with 0.1% formic acid.

Time (mins)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
8	100	0
9	0	100
15	0	100
16	100	0
20	100	0

Samples were injected with 50µL injection volume at a flow rate of 1.0mL/min. The optimum wavelength for both analytes (267nm) was determined using UV-Vis scanning spectroscopy. The optimised method was then transferred to a narrower bore 3.5µm particle size, 150 x 2.1mm Luna PFP reverse phase column for mass spectrometry application. The flowrate was adjusted to 0.3 mL/min and the injection volume was reduced to 20µL. A summary of the main parameters used are shown in Table 2.2.

Table 2.2 Parameters of LC for detection of famotidine and TPN

Mobile phase A	23:77 (v/v %) methanol/water with 0.1% formic acid
Mobile phase B	90:10 (v/v %) methanol/water with 0.1% formic acid
Flow-rate	0.3 mL/min
Wavelength	267 nm
Column type	3.5µm particle size, 150 x 2.1mm Luna PFP reverse phase column
Retention time (min)	2.45 for famotidine and 5.8 for TPN
Injection volume	20 µL
Run time	20 minutes

2.4 Mass Spectrometer

A Bruker Daltonics Esquire~LC ion trap MS with an electrospray ionisation interface at atmospheric pressure was used for MS analysis. MS conditions were optimised separately by direct infusion. Standard solutions (10mg/L) of each analyte were directly infused, using a Cole Parmer 74900 series syringe pump (Cole Parmer, Vernon Hills, IL, USA), into the mass spectrometer at a

flowrate of 300µL/h with a Hamilton 1710N gastight syringe. The analytes were monitored in positive mode. The parent ion response for TPN and famotidine were 242m/z (M+1) and 338m/z (M+1) respectively. MS conditions were automatically optimised using Bruker Esquire software for each analyte. The optimum intensities of each analyte were different for some focusing parameters therefore a compromised value was chosen. The precursor peak with the greatest intensity was fragmented using tandem MS and the most abundant product ion was chosen for monitoring of the tandem MS signal. The product ions for TPN and famotidine were 155m/z and 259m/z respectively and their likely structures are shown in Figure 2.2

Table 2.3 Mass spectrometer parameters and values.

Parameter	Default	TPN 242m/z	Famotidine 338m/z	Combined Method
Capillary Voltage	-4000V	-4500V	-4254V	-4000V
Endplate Offset	-500V	-718V	-752V	-1080.4V
Skim 1	35V	35.9V	15.0V	25V
Cap Exit Offset	60V	50V	50V	50V
Octopole	2.8V	2.77V	2.38V	301V
Octopole Delta	2.4V	2.39V	2.22V	2.34V
Trap Drive	55	45.57	33.06	37.2V
Skim 2	6V	7.62V	5.9V	6.4V
Octopole RF	150V	103.28V	213.93V	132.0V
Lens 1	-5V	-4.54V	-3.66V	-3.6V
Lens 2	-60.98V	-64.43V	-59.84V	-56.4V

The completed LC-ESI-MS/MS method for analysis used an Agilent 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a Bruker Daltonics Esquire-LC ion trap MS with an electrospray ionisation interface at atmospheric pressure (Bruker Daltonics, Coventry, UK). A Phenomenex narrow bore, 150 x 2.1mm Luna PFP reversed phase column with 3.5µm particle size was used for separation. The pentaflourophenyl propyl coated silica beads have a multiplicity of selectivity mechanisms including hydrogen bonding, dipole-dipole, aromatic and hydrophobic interactions, which make it ideal for separation of basic pharmaceuticals. A flowrate of 0.3mL/min and an injection volume of 20µL were used. The LC-ESI-MS/MS system was controlled using

Agilent Chemstation version A.06.01 and Bruker Daltonics Esquire Control version 6.08. Bruker Daltonics data analysis software was used for data analysis.

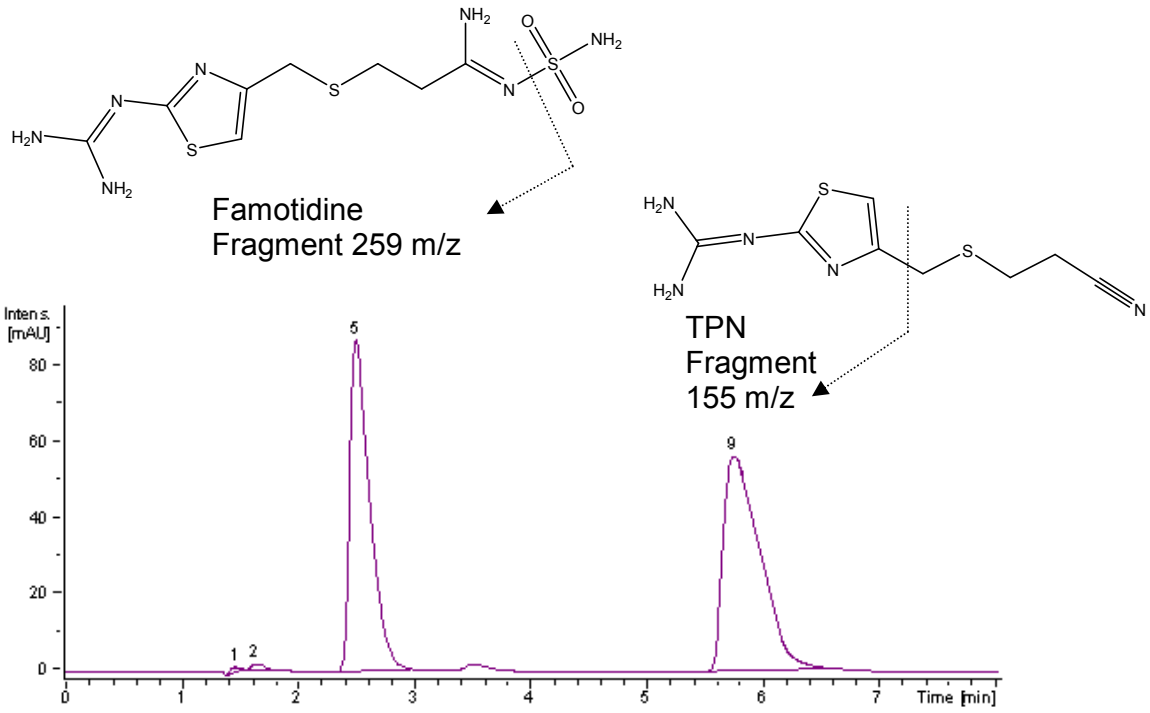

Figure 2.2 LC separation of famotidine and TPN and their corresponding daughter ions.

2.3 Solid Phase Extraction

Solid phase extraction (SPE) cartridges were used to pre-concentrate samples. Phenomenex Strata-X and Strata Screen and Strata X-C were investigated for recovery of the analytes. Strata X-C showed best recoveries (>80%). Strata X-C cartridges have mixed-mode selectivity which contains a reversed phase mode and a strong cation exchanger. As the cartridges are cation exchangers, the analyte must be positively charged in order for it to bind to the cartridge. As famotidine and TPN are weak bases, they were acidified using 1M phosphoric acid. Prior to extraction the solid phase cartridges were washed with three column volumes (6mL) of methanol followed by three column volumes of

water. Deionised water was spiked to a concentration of 40µg/L of each analyte and was acidified to pH 2.5 with 20µL of 1M phosphoric acid and brought to the mark (25mL) with deionised water. The analytes were passed through the solid phase extraction cartridges using vacuum. Cartridges were then washed with two column volumes of 0.1% phosphoric acid in water after the addition of the sample and dried for 5 minutes under vacuum. The cartridges were washed with two column volumes of methanol and eluted with 5% NH_3OH into 20mL amber centrifuge vials. The samples were dried using a MiVac Rotavaporator for 5h at 30°C and reconstituted in 1mL of mobile phase A.

To calculate the percentage recovery of each analyte, 1mL of working solution was added to a 25mL volumetric in triplicate and brought to the mark with deionised water, which had been acidified to pH 3 with 0.1% phosphoric acid. The analytes were then extracted by solid phase extraction and concentrated by a factor of 25. Three cartridges were loaded with acidified deionised water as a control, and were spiked with 1mL of working solution, post-extraction. These were dried on a MiVac Rotavaporator for 5h at 30°C to calculate any losses during drying. All six samples were assayed by LC-MS and compared against the working solution of 1mg/L. The concentration recovered was 93% ± 4%, of the initial concentration.

2.4 Water Sampling

Polypropylene bottles were used for the collection of wastewater samples at Astellas Pharma Co. Ltd., Dublin, Ireland and were transferred to amber glass bottles off-site. The amber bottles were silanised prior to sampling. Two sampling points were identified in the plant and are shown in (see figure 1.4). Sampling took place over a 6 week period (5th August 2009 to 16th September 2009) following a two week shutdown of the plant. Samples from both points were collected and transported to the laboratory. The samples were filtered

through Whatman glass fibre filters to remove suspended solids and adjusted to pH 3 using 5M phosphoric acid and samples were stored at 4^0C until analysed.

2.5 SuperPro Designer V 5.1

SuperPro Designer V 5.1® from Intelligen, Boston, MA, USA was used to model the production of famotidine and to estimate quantities of impurities produced, specifically, the reaction extent and completions were analysed. A process flow diagram was generated using information obtained about the equipment used in Astellas (see figure 1.4). Physical properties of the chemicals used in the production of famotidine were obtained from Astellas and were inputted to the model. The production of imidate was examined first using various permutations of reaction extents between the raw materials. Assumptions were made to elucidate what quantity of raw material was unreacted or converted to impurities, and what quantity of imidate is produced and converted to famotidine. Large amounts of data were generated and those which were far outside the actual range observed in Astellas were discarded. Several sensitivity analyses were performed on the model. The results from the sampling regime were used to corroborate the findings of the model.

Chapter 3

Production process of famotidine in Astellas Ltd., Ireland

3.1 Step 1 – Synthesis of imidate

420kg of the raw material TPN are dissolved in 1263L of mixed solvent, dioxane/methanol (2:1 v/v), in vessel VE-2100 (see Figure 3.1). TPN is known to have an impurity, A-5 (see Appendix A), which constitutes approximately 0 – 2.5% of TPN. There are eight known impurities which may be formed throughout the production process, named A-1 to A-8 and whose IUPAC names are listed in Appendix A. After mixing the solution of TPN for three days, HCl gas is passed through the solution for 14h to form imidate·HCl. The impurities produced at this stage in the process are known to be A-4 and A-3. Methanol reacts in a 1:1 reaction with TPN·HCl (see Figure 3.2).

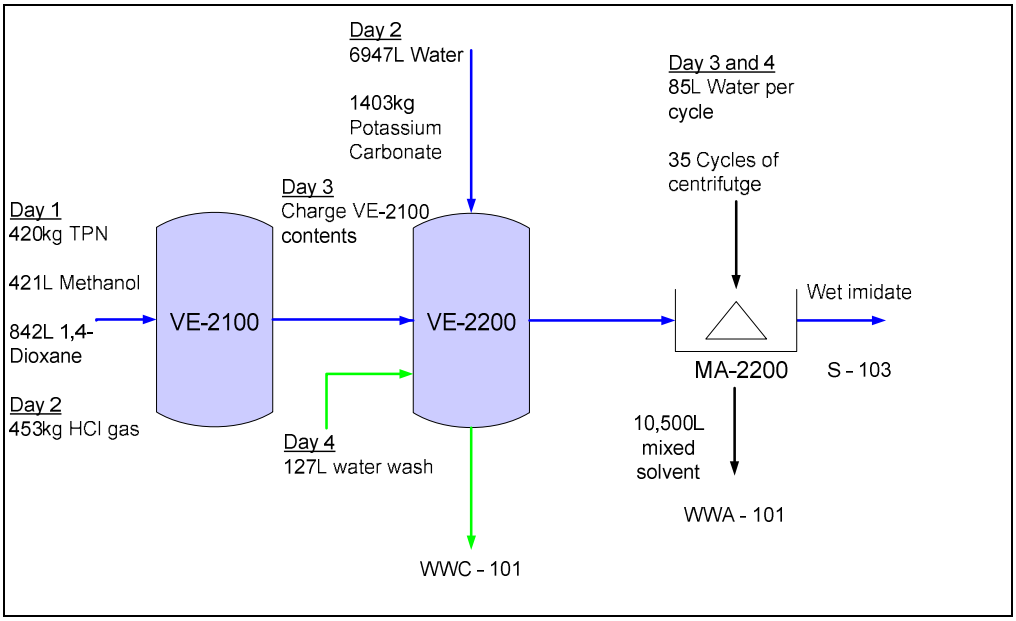

Figure 3.1 Formation of the intermediate imidate.

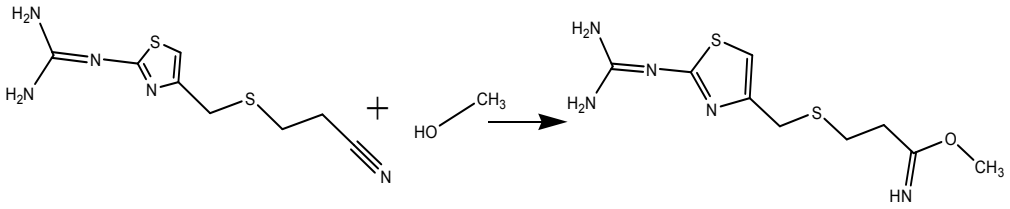

Figure 3.2 Protonation of TPN with HCl allows methanol to react and produce imidate·HCl (Astellas).

Imidate·HCl solution is transferred to the next vessel, VE-2200, where the hydrochloride molecules are removed and neutralised by 1403kg potassium carbonate dissolved in 6947L water (see Figure 3.1). The products of this reaction are free-imidate base, carbon dioxide, potassium chloride and water (see Figure 3.3).

$$2C_9H_{15}OS_2 \bullet HCl + K_2CO_3 \Rightarrow CO_2 + 2C_9H_{15}N_5OS_2 + 2KCl + H_2O$$

Figure 3.3 Imidate neutralisation reaction.

The solution is pumped to a basket centrifuge, MA-2200, and undergoes thirty cycles of centrifugation washing with 85L water for each cycle. The wastewater is then transferred to WWA – a holding tank which contains approximately 19% solvent and 81% water which subsequently is transferred for on-site thermal treatment (Ettarh, 2008).

The water washes remove inorganic compounds such as potassium carbonate and potassium chloride which is formed when hydrochloride reacts with potassium carbonate. A methanol wash then removes water from the imidate. No data regarding the compositions of the filtrate and retentate are available. Approximately 510 kg of wet imidate are produced which are equivalent to 455 kg dry imidate (Astellas Ireland Co. Ltd., 2006). The retentate slurry is

transferred to a hopper (VE-2320) in preparation for the next step in the process.

3.2 Step 2 – Synthesis of famotidine

349.9 kg of sulphamide are dissolved in 815L of methanol and 116.3L of triethylamine (TEA) in vessel VE-2300/2800. Wet imidate slurry from hopper VE-2320 is added to VE-2300/2800 in six aliquots over a period of 48h. Imidate and sulphamide react in a 1:1 reaction to form crude famotidine (CFM) and methanol (see Figure 3.4). Other compounds that may be produced in this reaction are A-7 and A-8, usually about 0.07% to 0.1% of crude famotidine yield. The yield of crude famotidine is approximately 74% from TPN. It is thought that this low yield is due to imidate degrading to impurities A-4 and A-7. However, no standard of any impurity (A-1 to A-8) was available to develop a method of detection.

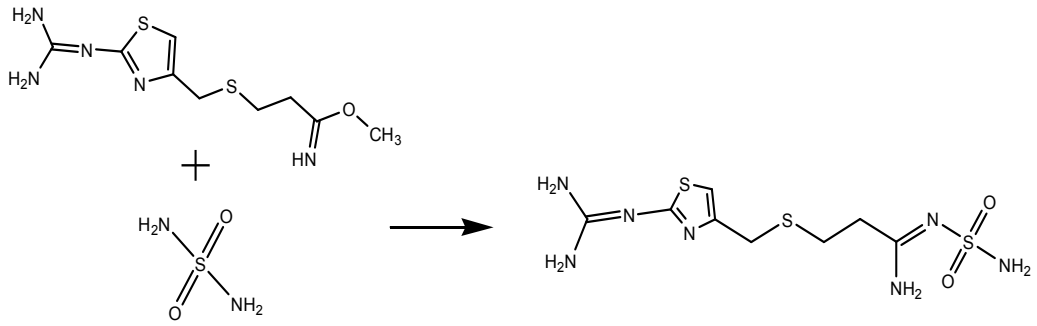

Figure 3.4 Imidate reacts with sulphamide to form famotidine.

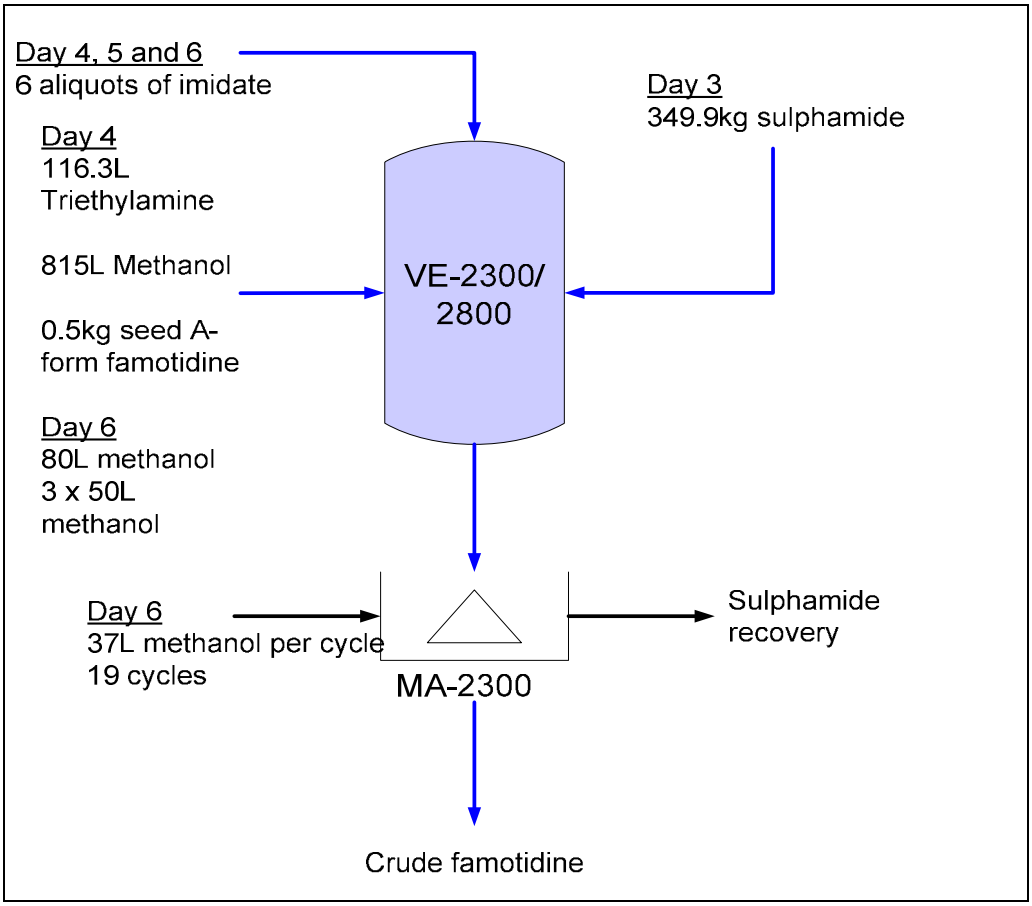

Figure 3.5 Vessel VE-2300/2800 is used for the reaction between sulphamide and imidate to produce famotidine.

The crude famotidine solution is seeded with 0.5kg of A-form famotidine crystals. This slurry is pumped to a centrifuge, MA-2300, and nineteen cycles of centrifugation are performed. Approximately 463kg of wet crude famotidine are present in the retentate. The dry weight of this is usually 413kg. The filtrate contains unreacted sulphamide, dissolved famotidine and impurities A-7 and A-8. The filtrate is transferred to a vacuum distillation column for recovering sulphamide. Filtrate is stored in a 3000L vessel at ambient temperature, and vacuum distillation is performed until a final volume of 750L is reached. 847L of ethanol are added and subsequently centrifuged in 9 cycles, washing with 50L of methanol per cycle. The typical recovery of sulphamide is 124 kg, from 245 kg initially. The waste methanol in the filtrate is transferred to WWA1 for thermal treatment.

3.3 Step 3 – Purification of semi-pure famotidine

Crude famotidine is stored in a hopper, VE-2420, until it is transferred to VE2400, where it is dissolved in 1308L of 1,1-dimethylformamide (DMF). 43.4L of TEA is added, which allows famotidine to crystallise and keep impurities in solution, during the seeding step. 1786L of water are added to prevent famotidine from re-dissolving, as famotidine is insoluble in water. The vessel is seeded with B-form famotidine crystals and famotidine molecules crystallise (see Figure 3.6).

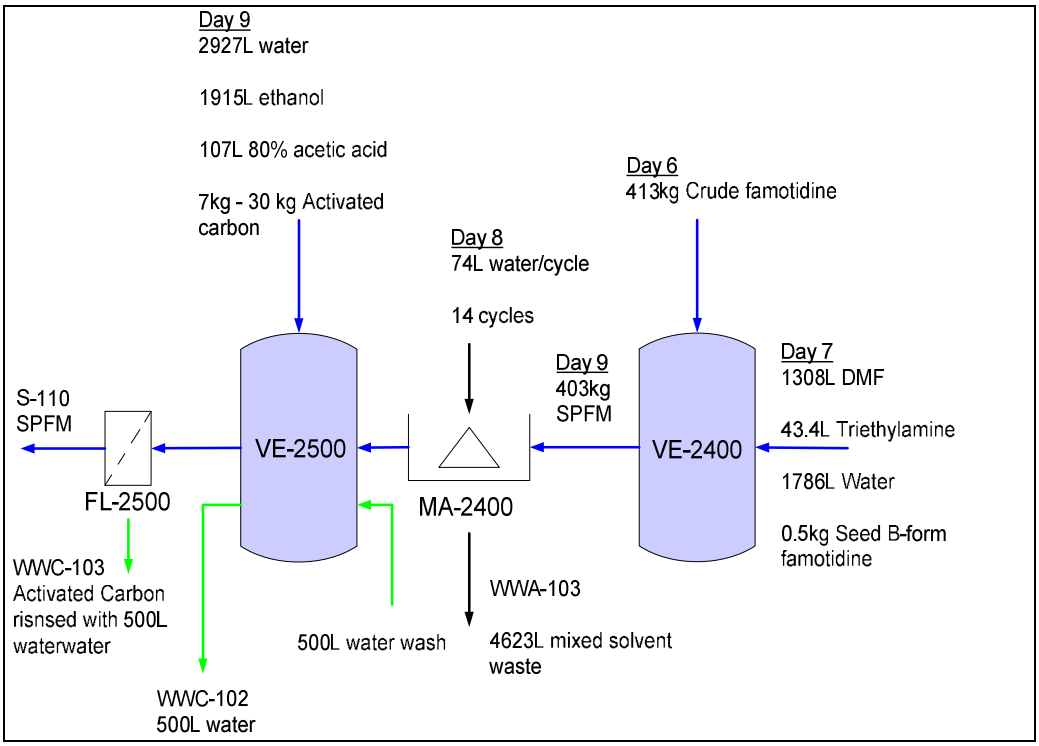

Figure 3.6 The 1st stage in purification involves crystallisation, centrifugation, dissolution and adsorption.

The slurry, known at this stage as semi-pure famotidine (SPFM) is pumped to centrifuge MA-2400, undergoing 14 cycles of 74L water washes. This water is transferred to WWA. The retentate is added to VE-2500, and is dissolved in a mixture of 2927L of water, 1915L of ethanol and 107L of acetic acid. Between 7kg and 30kg of activated carbon are added to the solution. The purpose of activated carbon is to remove impurities by adsorption. The quantity of

activated carbon is dependent on the amount of A-8 present following QC analysis of crude famotidine. The suspension of activated carbon and dissolved SPFM is filtered, removing all of the activated carbon. No data regarding the quantity of impurities or active pharmaceutical ingredients removed or remaining in solution are available.

3.4 Step 4 – Purification of final product

Depending on the polymorph required, the filtrate from the carbon filter containing dissolved SPFM is transferred to either VE-2600 for A-form or VE-4600 for B-form crystals (see Figure 3.7). 62.6kg of sodium hydroxide neutralises the acetic acid. The vessel is seeded with A-form famotidine to crystallise pure famotidine (PFM) and 394L of water are added to prevent famotidine from dissolving. The slurry is centrifuged for 25 cycles, with 60L of water and 30L of ethanol per cycle for A-form.

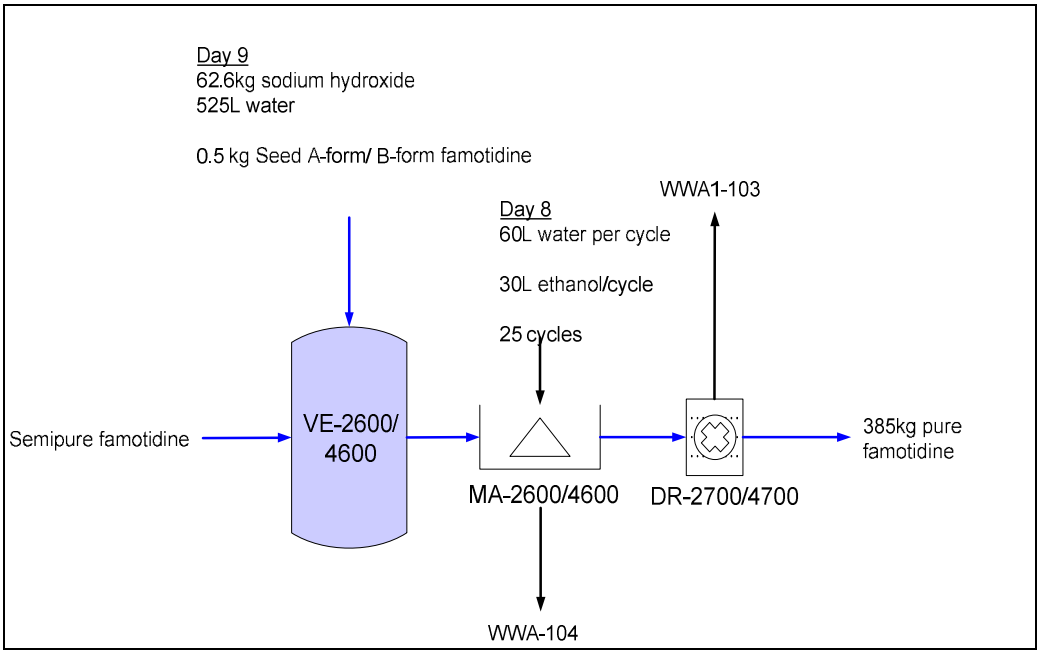

Figure 3.7 The final stage in the purification process of famotidine.

For the B-form route, the centrifuge is larger and 3 cycles of 346L of water and 172L of ethanol are performed. The retentate is then dried in a rotary dryer for 24h at less than 35˚C and approximately 385 kg of pure famotidine are

recovered. This equates approximately to a 65% yield. A theoretical one hundred percent molar stoichiometry of the process was calculated to yield 587kg of famotidine, if one hundred percent purity and completion of reactions are considered.

Chapter 4

Results and Discussion

4.1 Experimental results

Famotidine and TPN were detected at two sample points for each day of a six week sampling period (see Figure 4.1). The levels ranged from 0.8mg/L to 16.6mg/L in WWC1 and 0.27mg/L to 5.85mg/L in the pH adjust tank (see Table 4.1) for famotidine. Values of TPN detected ranged from 0.03mg/L – 0.44mg/L in WWC1 and from 0.01mg/L – 0.97mg/L in the pH adjust tank (see Table 4.1). For full tables of data see Appendix F. The highest concentration of famotidine (16.53 mg/L) was reported in the 4th week post shutdown, on the 2nd September in WWC1. This would equate to a mass of 1.653 kg of famotidine (see Table 4.2) and corresponds to 0.43% of the total average production of 385kg, assuming WWC1 was full and has a tank capacity of 100m^3. The tank capacity is a major assumption. It is not possible to quantify mass flows in the pH adjust tank as its capacity is not known and the tank has a weir and discharges by overflow on a continuing basis.

Table 4.1 Concentrations of famotidine and TPN in WWC1 and pH adjust tank.

Date	Conc. famotidine WWC1 (mg/L) (n=2)	Conc. TPN WWC1 (mg/L) (n=2)	Conc. Famotidine pH adjust (mg/L) (n=2)	Conc. TPN in pH adjust (mg/L) (n=2)
05-Aug	2.75	0.04	1.1	<LOQ
07-Aug	0.8	0.07	1.23	*
10-Aug	2.15	0.03	1.15	0.01
12-Aug	3.63	0.29	0.97	0.06
14-Aug	5.96	0.11	3.1	<LOQ
19-Aug	1.98	0.44	1.35	0.20
21-Aug	0.82	0.11	0.49	0.01
26-Aug	1.2	**	0.96	0.97
28-Aug	***	***	0.27	0.85
01-Sep	***	***	5.85	0.03
02-Sep	16.53	0.38	3.5	0.05
03-Sep	11.19	<LOQ	4.79	*
10-Sep	10.07	0.12	4.83	0.62
16-Sep	5.31	0.08	2.23	<LOQ

* Peak tailing occurred and samples were not quantified.
** Only one sample tested.
*** Values were not determined.

All samples were filtered through Whatman No. 3 glass fibre filters to remove suspended solids. The samples were then syringe filtered through 0.2µm nylon filters into glass amber HPLC vials. These were then analysed by LC-MS with the combined parameters (see Table 2.3). Famotidine was detected in each case. The presence of TPN was not as abundant in either tank and SPE was conducted on the samples to concentrate the analyte prior to LC-MS analysis. When analysing for TPN only, the optimised parameters for TPN were used (see Table 3.2). The samples were concentrated by a factor of 25 which gave responses of between 0.15mg/L to 22mg/L in WWC1. The percentage recovery of TPN through the SPE cartridges is 93% ± 4%. When the concentration factor and percentage recovery was taken into account, the measured values of TPN were between 0.007mg/L and 0.96mg/L (see Table 4.1). On five occasions, the quantity of TPN was not determined in the pH adjust tank (see Table 4.1). For two of these (7th Aug and 3rd Sep) peak tailing occurred during the chromatography stage of analysis and the concentration of TPN was not determined. On the other occasions (5th Aug, 14th Aug and 16th Sep) TPN was detected but their concentrations were below the limit of quantitation (0.016mgL)

In all cases except one, the concentration of famotidine was higher in WWC1 than in the pH adjust tank (see Figure 4.1). The model predicted that this would occur for each permutation (as described in section 4.4). The actual concentrations of famotidine (0.8mg/L to 16.6mg/L) are considerably below those modelled (0.879g/L and 0.954g/L). This may be explained on the grounds that other processes feed into both WWC1 and pH adjust tank. The wastewater from another pharmaceutical produced on-site is also transferred into the pH adjust tank. In addition water from the boiler house and cooling towers is pumped into WWC1 on a daily basis.

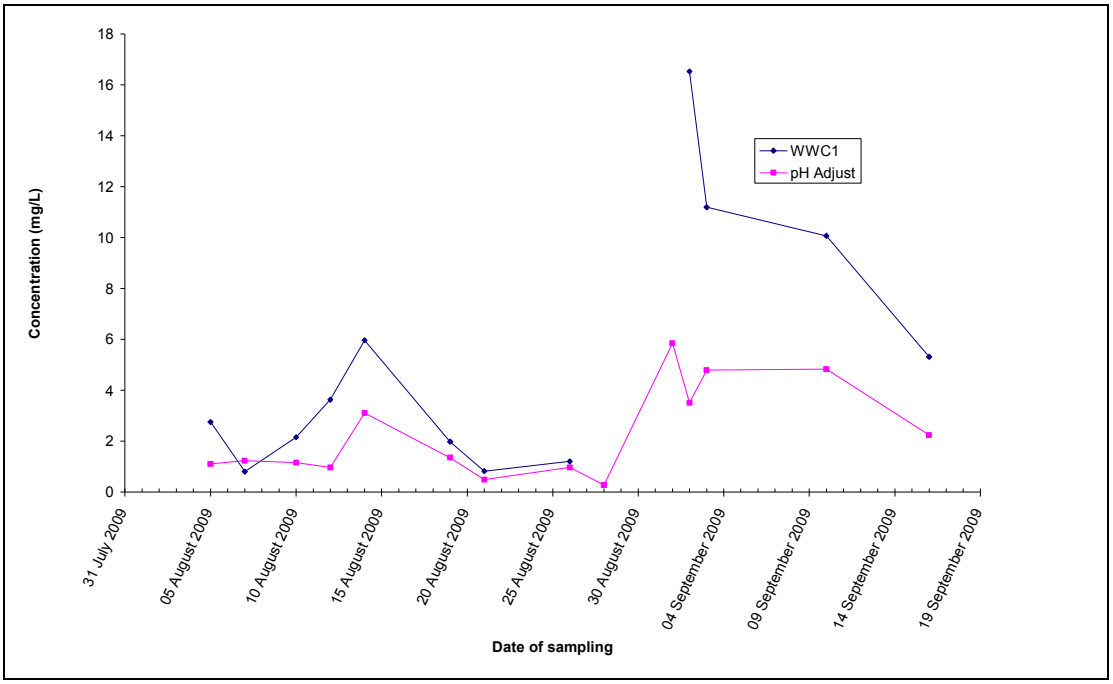

Figure 4.1 Timeline of famotidine sampling results in WWC1 (blue) and the pH adjust tank (pink).

It is not known what volumes of water are transferred to WWC1 and the pH adjust tank. Lower concentrations of TPN than famotidine were reported (0.007mg/L and 0.96mg/L) in WWC1 (see Table 4.1) in all samples. This was predicted by the model because the quantity of famotidine present in VE-2300/2800 is much larger than TPN (approximately 26 times) and therefore a larger quantity of famotidine is likely to be transferred to the wastewater treatment plant. In all but four cases (12th August, 19th August, 26th August and the 2nd September), TPN was found to be in a higher concentration in WWC1 than in the pH adjust tank. This is contrary to what is predicted in the model, in which TPN was shown to have higher concentrations in the pH adjust tank. This anomaly may be explained by a higher dilution factor of TPN by other water entering the pH adjust tank.

The left hand columns of Table 4.2 outline a timeline of the water tank washes which are transferred to WWC1. The estimated quantity of both famotidine and TPN in WWC1 are shown in the green columns. These predictions are based on the assumption that WWC1 is full and has a capacity of 100m^3. Such

estimates were not possible for the pH adjust tank but show the concentrations of famotidine and TPN on each sample day.

The WWC1 tank automatically empties when it reaches a point. This occurs continuously, emptying several times per week. As the tank emptied automatically, it was difficult to know what volume of wastewater was in WWC1 when sampling the tank. The process washes could not be linked to the concentrations of TPN and famotidine in WWC1. A sampling point after each of the washes and centrifuges would be beneficial in monitoring concentrations of the analytes rather than measuring the concentrations in wastewater tanks. The lost quantities of both famotidine and TPN from the process could not be back calculated as a result of this. Ideally, one would sample at each of the centrifuges to obtain accurate data regarding the composition of the filtrate. The closest point to obtain samples of filtrate is at the pH adjust tank, which is after solvent recovery.

Table 4.2 LC-MS results and approximate losses of both analytes from the process.

Date	Washes VE-2200 (L)	Washes VE-2500 (L)	Total Washes (L)	Estimated Capacity of WWC1 (L)	Conc. famotidine WWC1 (mg/L)	Estimated quantity of famotidine (kg)	Conc. TPN WWC1 (mg/L)	Estimated quantity of TPN (kg)
05-Aug	127	-	127	100,000	2.75	0.275	0.04	0.004
07-Aug	127	-	127	100,000	0.8	0.8	0.07	0.002
10-Aug	127	-	127	100,000	2.15	0.215	0.03	0.0001
12-Aug	127	500	627	100,000	3.63	0.363	0.29	0.009
14-Aug	-	-	0	100,000	5.96	0.596	0.11	0.004
17-Aug	-	500	500	-	-	-	-	-
18-Aug	127	-	127	-	-	-	-	-
19-Aug	-	-	0	100,000	1.98	0.198	0.44	0.014
21-Aug	-	-	0	100,000	0.82	0.82	0.11	0.004
23-Aug	127	-	127	-	-	-	-	-
24-Aug	-	500	500	-	-	-	-	-
26-Aug	127	500	627	100,000	1.2	0.12	0.43*	0.014
28-Aug	-	-	0	100,000	**	-	**	-
31-Aug	-	500	500	100,000	-	-	-	-
01-Sep	127	-	127	100,000	**	-	**	-
02-Sep	-	-	-	100,000	16.53	1.653	0.38	0.012
320,000	127	-	127	100,000	11.19	1.119	<LOQ	<LOQ
-	-	500	500		-	-	-	-
05-Sep	-	-	-	-	-	-	-	-
06-Sep	127	-	127	-	-	-	-	-
09-Sep	127	500	627	100,000	-	-	-	-
10-Sep	-	-	-	100,000	10.07	1.007	0.12	0.004
14-Sep	127	500	627	-	-	-	-	-
16-Sep	-	500	500	100,000	5.31	0.531	0.08	0.003

* This sample was not performed in duplicate. ** Not determined.

4.2 SuperPro Designer v5.0

SuperPro Designer V 5.1® from Intelligen, Boston, MA, USA was used to model the production of famotidine. This software is used to model chemical processes and monitor their performance. It allows the user to select various unit operations, such as reaction vessels, distillation columns, chromatography columns, centrifuges etc. Specific data about a process can be inputted, for example, chemical reactions, reaction extents, quantity of by-products created and crystallisation efficiency. SuperPro Designer was used to model the production of famotidine and to estimate quantities of impurities produced. Specifically, the reaction extent and completions were varied in a series of modelling steps in order to elucidate what quantity of raw material is unreacted or converted to impurities, and what quantity of intermediates is produced. Two production batches were studied (7th April 2008 and 4th August 2009) and data taken on site during these processes were used to create the SuperPro Designer model. These data were compared with average values of previous production batches and showed no significant deviation.

Various assumptions were made in order to provide a closely fitting model with the real process. Assumptions can also eliminate unnecessary calculations and by omitting data which were far outside the likely range, the calculations were more focussed. The SuperPro Designer model created was split into four parts: imidate production (see Figure 3.1), crude famotidine production (see Figure 3.4), semi-pure famotidine purification (see Figure 3.6) and final pure famotidine (see Figure 3.7). The two sampling points at the Astellas facility are at WWC1 and the pH adjust tank (see Figure 4.2).

The assumptions which influenced the model outputs to the greatest degree were: (i) the purity of TPN (97% - 100%), (ii) the conversion of TPN to imidate (90% - 95%) and (iii) the conversion of imidate to crude famotidine (76% -

78%). Assumptions which fit the average production batch best are shown in Appendix B. The model works well in predicting the percentage composition of solvents in waste streams (Ettarh, 2008) (see Appendix G). From the information provided by Astellas, the model showed a reasonably good relationship with the quantity of product predicted to be produced and product lost to waste streams. More information is required however, regarding aspects of the other processes on site, and by how much they are diluting the compounds of interest in waste streams. Up to 12.84kg of famotidine are predicted to be lost during the purification process.

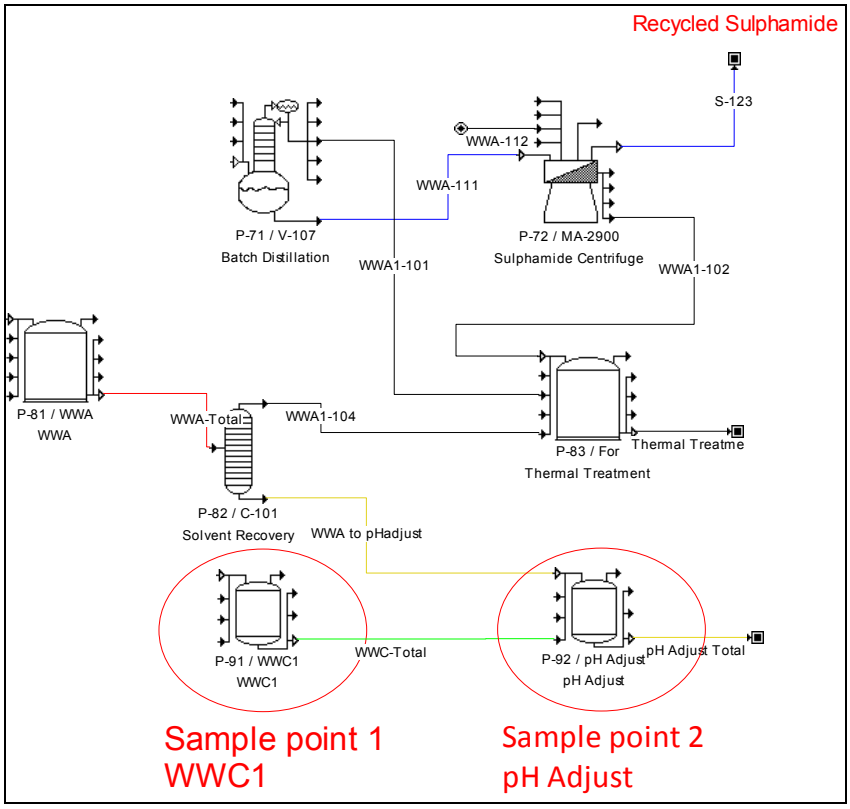

Figure 4.2 Wastewater streams of the production process which displays the sampling points.

In the first step in the process, the intermediate imidate is produced. A range of values for the purity of TPN, the quantity of imidate formed, the amount of impurities produced and the amount of material which is retained in the first centrifuge, MA-2200 was investigated using baseline data obtained from

Astellas. The baseline data indicate that during this process, 455kg of imidate with 95% purity are produced. Therefore any predicted value outside the range of 450kg – 460kg was rejected from the model. Using SuperPro designer, VE-2200 was programmed to retain 0.1% of the total volume of the mother liquor. This would simulate residue adhering to the walls of the tank. The tank was then washed with 127L water and transferred to WWC1. The values used for components in three of the streams in the model were monitored: the filtrate to WWA (WWA-101), the retentate continuing with the batch (S-103) and the 127L water wash of VE-2200 (WWC-101). Any calculated values that lay outside the range of 420kg – 430kg of pure imidate observed in Astellas were discarded. 25kg of impure imidate are impurities or unreacted TPN and the weights of each are accounted for in the model. SuperPro calculates the composition of each stream and presents data in tabular format. An example of stream composition is shown in Table 4.3.

Table 4.3 Composition of stream S-103 following centrifugation in MA-2200.

Component	Flowrate (kg/batch)	Mass Composition (%)	Conc. (g/L)
A-2	0.45	0.09	0.99
A-3	4.24	0.85	9.39
A-4	4.49	0.90	9.93
A-5	3.99	0.80	8.82
Imidate	428.58	85.60	948.65
Methanol	45.67	9.12	101.08
TPN	13.29	2.65	29.42

This example is based on the assumptions that: (i) TPN is 99% pure (415.8kg TPN and 4.2kg A-5), (ii) the reaction between TPN and methanol is 92% and (iii) 99% of imidate is in the retentate (S-103) post centrifugation. One percent of imidate is assumed to be lost in the centrifugation process and is transferred to stream WWA-101 (see Table 4.4).

Table 4.4 Wastewater stream WWA-101 post centrifugation in MA-2200.

Component	Flowrate (kg/batch)	Mass Composition (%)	Conc. (g/L)
A-2	8.51	0.07	0.72
A-3	0.22	0.00	0.02
A-4	0.24	0.00	0.02
A-5	0.21	0.00	0.02
Ammonia	0.88	0.01	0.07
Carbon dioxide	64.87	0.53	5.47
Dioxane(1,4)	879.49	7.20	74.17
Imidate	4.33	0.04	0.37
Potassium chloride	337.82	2.76	28.49
Methanol	291.18	2.38	24.56
Potassium carbonate	1088.47	8.91	91.80
TPN	3.32	0.03	0.28
Water	9542.68	78.08	804.78

4.3.1 SuperPro Designer modelling of imidate production

The impurity A-5 is known to be present in TPN and therefore the purity of TPN was included as a variable. The purity of the raw material is an important factor in analysing the overall process. With purity below 100%, the model becomes more complicated due to the presence of impurity A-5. Preliminary values for purity of TPN ranged from 70% - 100%. Further information from Astellas revealed that the range in purity was between 97.5% and 100% for TPN. A-3 and A-4 are known to form during the reaction of TPN and methanol and are found in impure imidate. However it was not known what quantity of either impurity was in impure imidate.

By analysing the data at this stage in the process, important assumptions in the imidate production stage could be verified. The TPN purity is thought to be between 97% and 100% with a reaction extent of between 90% and 95%.
The purity of imidate after MA-2200 is usually 95%. The 5% impurities are made up by unreacted TPN and small quantities of A-3, A-4 and A-5. Unreacted TPN reacts with water to form A-3. As water is abundant at this

stage in the process (6947L), it was assumed that 50% of TPN would react with water to create A-3. A-3 reacts with water to form A-2, an inorganic compound. It was assumed that the conversion rate of A-3 to A-2 was 50% and a further 25% was assumed to be converted to A-4. The quantities of these impurities, along with that of unreacted TPN were important when considering the efficiency of the process.

The dry weight of imidate was modelled to be between 450kg and 460kg. Routine HPLC analysis in Astellas indicates that the purity of dry imidate is 95%, thus approximately 25 kg of imidate are impurities. Modelling revealed that the purity of TPN was between 97% and 100% and the conversion rates of imidate production were between 90% and 95% (see Table 4.5). Therefore the quantity of pure imidate produced was modelled to be between 420kg and 430kg. All values which predicted the dry weight of impure imidate to be outside the range of 450kg – 460kg were discarded. Of these valid predictions, those which predicted pure imidate to be outside the range 420kg – 430kg were also discarded.

Table 4.5 The values used for the assumptions made to model Step 1 using SuperPro Designer.

Assumption	Investigated values (%)	Values modelled (%)
TPN purity	70 - 100	97 - 100
Reaction completion	70 - 100	90 - 100
TPN in retentate	0, 70, 80, 90, 99, 100	80
Imidate in retentate	99 and 100	99 and 100

Once imidate is produced in VE-2200, it is transferred to a centrifuge, MA-2200. Ninety five percent of A-2 was assumed to be washed away in the filtrate, WWA-101, as it is an inorganic compound. All other inorganic compounds were assumed to be removed to the filtrate. Twelve permutations regarding the fate of TPN and A-5 in the centrifuge were examined. These were investigated as it is currently unknown what fate these compounds have in the centrifuge (i.e. whether either compound is removed in the filtrate or remains in the retentate). 80% retention of TPN

keeps the quantity of impure imidate between 450kg and 460kg, whereas higher retention rates of TPN result in quantities of impure imidate above the accepted range. TPN is highly insoluble in water and has large crystal size, (see Figure 1.3) which would lead to the assumption that unreacted TPN remains with impure imidate. All unreacted TPN is assumed to be present with A-5 the impure imidate.

Eighty eight data points were generated and this number had to be reduced. Any data points generated which predicted TPN purity to be lower than 97% were discarded. Elimination of the irrelevant data points was necessary in order to manage the data. The data which were within the range and were consistent with the assumptions are presented in Table 4.6. This represents permutations of the aggregate masses of TPN, imidate and impurities (A-2 to A-5), all of which are between 450kg and 460kg

Stream S-103 contains imidate (see Table 4.3), which accounts for 94.1% of the solid material in the stream. The remaining 5.9% of solid material comprises impurities (A-2, A-3, A-4 and A-5) and unreacted TPN. Values examined for the retention of TPN post centrifugation were 100%, 99%, 90%, 80%, 70% and 0%. The actual conversion of TPN to impure imidate (94 % - 96 % pure) is approximately 95% and an assumed retention of 80% of TPN corresponds to the actual value of impure imidate. Sixty seven permutations of impure imidate were modelled generating values between 450kg and 460kg. These data were further reduced to 30 points, as only crude imidate with a quantity of pure imidate which was between 420kg – 430kg was kept. These values were brought forward to the next stage for further analysis. The subsequent washes of VE-2200 were recorded and are included in the mass balance for WWC1.

Table 4.6 Process inputs for imidate formation and post MA-2200 stream components

Assumptions modelled			Process stream S-103 components (kg) post MA-2200						
TPN purity (%)	**Reaction Completion (%).**	**Imidate in retentate (%)**	**A-2**	**A-3**	**A-4**	**A-5**	**Imidate**	**TPN**	**Total impure imidate**
100	91	99	0.51	4.82	5.10	0.00	428.20	15.26	453.88
100	91	99	0.51	4.82	5.10	0.00	428.20	15.10	453.73
100	90	100	0.57	5.35	5.66	0.00	427.77	16.95	456.31
100	90	100	0.57	5.35	5.66	0.00	427.77	16.78	456.14
100	90	99	0.57	5.35	5.66	0.00	423.49	16.95	452.03
100	90	99	0.57	5.35	5.66	0.00	423.49	16.78	451.86
99	92	99	0.45	4.24	4.49	3.99	428.58	13.43	455.16
99	92	99	0.45	4.24	4.49	3.99	428.58	13.29	455.03
99	91	100	0.50	4.77	5.05	3.99	428.20	15.10	457.61
99	91	100	0.50	4.77	5.05	3.99	428.20	14.95	457.46
99	91	99	0.50	4.77	5.05	3.99	423.92	15.10	453.33
99	91	99	0.50	4.77	5.05	3.99	423.92	14.95	453.18
99	90	100	0.56	5.30	5.61	3.99	423.49	16.78	455.73
99	90	100	0.56	5.30	5.61	3.99	423.49	16.62	455.57
98	93	99	0.39	3.67	3.89	7.97	428.86	11.63	456.41
98	93	99	0.39	3.67	3.89	7.97	428.86	11.51	456.29
98	92	100	0.44	4.20	4.44	7.97	428.53	13.29	458.88
98	92	100	0.44	4.20	4.44	7.97	428.53	13.16	458.75
98	92	99	0.44	4.20	4.44	7.97	424.25	13.29	454.59
98	91	100	0.50	4.72	5.00	7.97	423.87	14.95	457.02
98	91	100	0.50	4.72	5.00	7.97	423.87	14.80	456.87
97	95	99	0.27	2.60	2.75	11.96	433.61	8.22	459.41
97	95	99	0.27	2.60	2.75	11.96	433.61	8.14	459.33
97	94	99	0.33	3.12	3.30	11.96	429.05	9.87	457.61
97	94	99	0.33	3.12	3.30	11.96	429.05	9.77	457.52
97	93	100	0.38	3.64	3.85	11.96	428.77	11.40	459.99
97	93	99	0.38	3.64	3.85	11.96	424.48	11.51	455.82
97	93	99	0.38	3.64	3.85	11.96	424.48	11.40	455.70
97	92	100	0.44	4.16	4.40	11.96	424.16	13.16	458.26
97	92	100	0.44	4.16	4.40	11.96	424.16	13.02	458.13

The left hand side of the Table 4.6 (columns 1 to 3) displays the assumptions modelled with all permutations. The corresponding outputs of these assumptions are listed on the right hand side of the table (columns 4 to 9). The aggregate dry weight of impure imidate is presented in column 10. The methanol component of S-103 is omitted from the table as only the dry value is of interest. The total impure imidate aggregate values shown are within the range of 450kg and 460kg.

The quantity of pure TPN is inversely related to the quantity of A-5 – the impurity commonly found in TPN. It was assumed that A-5 was inert and proceeded through the process unreacted and 95% remained in the retentate following the first centrifuge, MA-2200. Unreacted TPN and A-5, along with other impurities, A-2, A-3 and A-4 contribute to the weight of impure imidate post-centrifugation, and this has been accounted for in the model (see Table 4.6).

4.3.2 SuperPro Designer modelling of TPN fate in WWC1

The quantities of TPN lost to WWC1 are predicted to range from 0.014 kg to 0.021kg. The 127L water washes of VE-2200 and subsequent discharge to WWC-101 are the only routes whereby TPN enters WWC1 (see Figure 1.4). Neither of the other water washes (streams WWC-102 and WWC-103) which feed into WWC1 contains traces of TPN. A much larger amount of TPN is discharged to WWA-101 in the cake washes of MA-2200 and MA-2300/2800. The very low concentrations of TPN predicted to be in WWC1 (0.022g/L – 0.032g/L) are approximately a factor of 100 more than those in actual samples. This is similar in magnitude to the differences between the model predictions for famotidine concentration in WWC1 and the experimental data.

The water which is used to wash tank VE-2200 (see Figure 3.1) is transferred to holding tank WWC1 where actual water samples were taken (see Figure 1.4). It is predicted by the model that between 0.01kg and 0.02kg of TPN is transferred to WWC1. This accounts for 0.1% of unreacted TPN in VE-2200. WWC1 is used as a tank wash receiver at two times throughout the process, but only this stage is predicted to contribute to the presence of TPN in WWC1. Samples were taken immediately after a two week shut down of the plant. It was expected that there would be zero quantities of TPN in the samples. However, residual quantities of TPN were recorded (see Table 4.1).

Based on an estimated tank size of 100m^3, this equates to a mass of 44g of TPN.

4.3.3 SuperPro Designer modelling of TPN fate in WWA

The quantities of TPN which are removed in the centrifuge MA-2200 to stream WWA-101 are predicted to be between 2.878kg and 4.196kg (see Table 4.1). The filtrate (WWA-103) from centrifuge MA-2400 is estimated to contain between 0.115kg and 0.168kg TPN and is stored in WWA. The model predicts that the aqueous fraction of WWA represents 65% of the total 18775L per batch. The liquid from WWA is distilled and the distillate is transferred for thermal treatment and the aqueous fraction is sent to the pH adjust tank. The pH adjust tank contains the condensate from the solvent recovery and the contents from WWC1. The predicted quantities of TPN in the pH adjust tank (3.008kg – 4.384kg) are much greater than those in WWC1 (0.014kg – 0.021kg).

The water volume of WWC1 (627L) is much lower than the volume of condensate from the solvent recovery step (13,840L) which means the concentrations of TPN predicted in WWC1 (0.022g/L – 0.032g/L) and pH adjust (0.085g/L – 0.122g/L) are of the same order of magnitude. Approximately 73% of the unused TPN (up to 16.59 kg per batch) is predicted by the model to then go to thermal treatment.

4.4 SuperPro Designer modelling of crude famotidine production

The reaction of imidate and sulphamide creates famotidine. From batch studies and information provided by Astellas it is known that yield of crude famotidine is approximately 74%. This does not take into account the impurities that are transferred from MA-2200 (A-3, A-4 and A-5) or any unreacted TPN. Other impurities are formed during the reaction between imidate and sulphamide. Impurities A-7 and A-8 are formed at this point in

the process. The percentage of A-8 present in crude famotidine determines the amount of activated carbon used to purify the crude famotidine at a later step. From information received from Astellas, A-7 is unstable, and is converted to A-8. However, programming SuperPro to perform this reaction proved difficult, and an alternative reaction was made. 0.1% of famotidine reacts with imidate in a 1:1 ratio, to produce an equimolar quantity of A-8, ammonia and methanol. This equates to about 0.06% to 0.07% of the yield of crude famotidine. These quantities of impurities had to be taken into account and were included in the permutations investigated. The purity of crude famotidine is usually 95% and for the model a range of 91% - 96% was examined. The dry weight of crude famotidine is approximately 413kg. The range of crude famotidine accepted for the model was between 405kg and 420kg. The conversion rate of imidate and sulphamide to crude famotidine was initially modelled between 10% and 100%. The assumptions made are shown in Table 4.7. Impurities that are found at Astellas at this stage are A-7 and A-8.

Table 4.7 Values used for the assumptions made to model the production of crude famotidine using SuperPro Designer.

Assumption	Preliminary values	Values modelled
Reaction Completion	10% - 100%,	70% - 80%
Crystallisation	94%, 96%, 98%, 99% and 100%	99%

All values outside 70% and 80% were far from those observed in Astellas and were discarded. The range of 70% to 80% was narrowed further to between 74% and 78%. After the reaction, the reactor is seeded with A-form crystals. It is assumed that the efficiency of crystallisation is 99% and that some of the impurities are also crystallised. It is assumed that only 1% of the unreacted imidate remains with the crude famotidine. The permutations of this step reveal that the quantities of unreacted imidate ranged from 92.54kg and 110.845kg, a majority of which is eventually transferred for thermal treatment. The reaction of imidate and sulphamide in VE-2800 has a low

yield, typically 74%, and it is at this stage that most of the losses of product occur. The reason the yield is low (74%) can be ascribed to two possibilities: either the breakdown of imidate to impurities A-7 and A-8, or imidate is unreacted and is eluted in the filtrate. In either case, there is a large quantity of material in excess of 100kg per batch being sent to the sulphamide recovery facility. At the recovery step, the distillate is not used any further in the process, and is transferred to WWA1 for thermal treatment. It would be of interest to sample the composition of the waste stream at this point, but there is no sampling point here.

4.4.1 SuperPro Designer modelling of famotidine fate in WWC1

The largest loss is predicted to occur during centrifugation at MA-2600/4600. Famotidine is first produced in VE-2300 when imidate reacts with sulphamide. After this reaction the first water wash of a reactor which is transferred to WWC1 happens in VE-2500. At this point famotidine has been dissolved in water, ethanol and acetic acid. Powdered activated carbon is added to remove impurities. It is assumed that 0.1% of the tank contents adhere to its walls which is then washed with 500L of water which goes to WWC1 via stream WWC-101. Between 0.384kg and 0.405kg of famotidine are lost at this point. The dissolved famotidine and powdered activated carbon are passed into a bag filter where the product is in the filtrate. Although the purpose of this process is to remove impurities, it is assumed that famotidine is also adsorbed.

Research investigating the properties of various activated carbons is being carried out in the School of Biotechnology in DCU. A preliminary study of the powdered activated carbon used by Astellas indicates high adsorptive properties. Isotherms investigating adsorption of famotidine with concentrations of 0-50mg/L in 50mL of water (pH4) with 0.1g of activated carbon were performed. In all instances the famotidine was completely removed. From these experiments the activated carbon is calculated to have

a maximum adsorbance capacity for famotidine of approximately 110mg/g. Astellas use between 7kg and 30kg of activated carbon per batch and an average of 18kg was used for the model. It is assumed that 0.5% of dissolved famotidine is adsorbed and removed from the mother liquor. Up to 2.201kg of famotidine are predicted to be removed from the process stream. The carbon is rinsed with water and packaged for off-site incineration. Ten percent of the famotidine is assumed to be removed during rinsing and the rinse water passes eventually into WWC1. This 10% loss of famotidine has been included in each modelled batch. However the volume of water is unknown and not included in the model.

As is the case with TPN, more famotidine (10.094kg) is predicted by the model to be present in the condensate of the solvent recovery stage than the quantity in WWC1 (0.625kg). The concentrations predicted to be in the pH adjust tank and WWC1 were 0.332g/L - 0.35g/L and 0.879g/L - 0.954g/L, respectively. In this case, the wastewater from WWA dilutes the famotidine to a lower concentration than what is estimated to be present in WWC1.

4.4.2 SuperPro Designer modelling of famotidine fate in WWA

Quantities of famotidine which were predicted to be in WWA are lost by means of cake washing in centrifuges MA-2400 and MA-2600/4600. MA-2400 separates B-form famotidine crystals from the process stream and the cake is washed with water (14 cycles of 75L/cycle) into waste stream WWA-103 (see Figure 3.6). Up to 4.224kg of famotidine are predicted to be lost. In the last centrifuge of the process (MA-2600/4600) as much as 6.013kg are estimated to be washed into the filtrate (WWA-104) (see Figure 3.7). As mentioned above, the contents of WWA undergo solvent recovery. The quantities of famotidine in the condensate range from 9.58kg to 10.09kg.

4.5 Sulphamide recovery

Following centrifugation in MA-2300, the filtrate is processed to recover sulphamide and recycle it back into VE-2300. The filtrate of MA-2300 is distilled and the condensate is centrifuged in MA-2900. The unreacted imidate is not assumed to have crystallised in the seeding process of VE-2300/2800 and therefore remained dissolved. The cake is washed with methanol and the model predicts that up to 145kg of sulphamide are recycled. Only 4kg of famotidine are lost to thermal treatment which is in contrast to TPN where up to 20.77kg are lost to this treatment.

The SuperPro designer model predicts a larger amount of famotidine than TPN present in WWC1. This is observed in the actual process in all cases of sampling. The quantity of famotidine produced is approximately 26 times that of unreacted TPN available. Therefore it is reasonable to assume that more famotidine than TPN will be lost. The actual wastewater analysis indicates low concentrations of both analytes but when one considers that the capacity of WWC1 is approximately 320m^3, significant quantities of each analyte are involved. In a homogenous mixture this could equate to up to 1.653kg of famotidine on the 2nd September, and up to 0.044kg TPN on the 19th August. WWC1 does not only store water from the famotidine process. More water is used by the boiler house and cooling towers. This contributes to a dilution factor of both analytes. An investigation into water usage in Astellas was carried out in 2005 which noted that the famotidine process uses 118m^3 industrial water per week equalling 6.7% of the overall consumption (Brookes and Duffy, 2005). The model predicts approximately a 2kg loss of famotidine following a wash down of VE-2500.

As the model accurately predicts the relative quantities of both TPN and famotidine in wastewater streams, then some credence can be given to the predicted quantities of other components in the process. For example, the quantity of the intermediate compound imidate, which reacts with sulphamide to form famotidine, is predicted to be in excess of 100kg after this step. This is one of the most abundant non-solvents in the process, after

famotidine, sodium acetate and potassium chloride. This may be of importance to Astellas as it may be possible to recycle it, thereby reducing costs. It may also be of significance when one considers the possibility of the introduction of more stringent regulations in the Water Framework Directive.

The contents of WWC1 are transferred to the second sampling point, the pH adjust tank. Other process waters are also transferred to this tank, which in turn are expected to dilute both analytes. This is the case for famotidine as all concentrations analysed are lower in this tank. TPN generally has a lower concentration in the pH adjust tank, except for the samples taken on the 26th August and the 10th September. On these occasions there was a large difference in sample responses using LC-MS (see Appendix F). The corresponding WWC1 sample was not tested in duplicate on the 26th August and for WWC1 on the 10 September the sample responses differed hugely (22475 and 8413, see Appendix F). Therefore these results may not be accurate. When analysing TPN from the pH adjust tank the extracted ion chromatogram of TPN showed tailing factor of more than 1.5 in some instances. This is likely due to the matrix of the sample. This phenomenon did not occur in the samples from WWC1. As the pH adjust tank is fed by another pharmaceutical process, it is assumed that this caused the TPN peak to tail. The pH adjust sampling point operates by overflowing into a lagoon. It does not get emptied and remains at the same level all of the time. No data was available to determine either the inflow or outflow of wastewater in the system. Therefore a mass balance of this point was not possible.

4.6 Model Steps 3 and 4 - Purification of famotidine

Data from the crude famotidine production step were brought forward to the purification stage. The assumption that there was a loss of product in the crystalliser VE-2400 was investigated. Crystallisation efficiency values of 94%, 96%, 98%, 99% and 100% were input into SuperPro. As there was only one

assumption at this stage of the model, it was coupled with those of step 4: adsorption of material to activated carbon in VE-2500, and crystallisation of pure famotidine in VE-2600/4600.

Efficiency values for both crystallisation steps were assumed to be between 94% and 100%. The amount of semi-pure famotidine (SPFM) removed by adsorption to activated carbon was examined. The role of the activated carbon is to remove residual impurities from the SPFM. These impurities have similar structures to the final product so it is likely that pure famotidine is also adsorbed and removed from the process. The values examined are shown in Table 4.8.

Table 4.8 Values used for the assumptions made to model the purification of famotidine using SuperPro Designer.

Assumption	**Percentages Investigated**
Crystallisation in VE-2400	95%, 96%, 97%, 98%, 99%, 100%
Activated carbon removal of SPFM	0.1 %, 0.5% and 1%
Crystallisation in VE-4600	95%, 96%, 97%, 98%, 99%, 100%

The average quantity of pure famotidine recovered by Astellas is 385kg. A range of 375kg to 395kg was applied to the model. Any value outside this was not considered. From these acceptable data, it was elucidated that the crystallisation that occurs in each of the reactors VE-2400 and VE-4600 was between 98% and 100%.

Chapter 5

Conclusions

5.1 Conclusions

There was no sample point for wastewater closer to the process than WWC1. A model was constructed using information regarding the famotidine production process and in consultation with key personnel from Astellas. No concentrations of impurities, intermediates, raw materials or products in wastewater streams in the plant had previously been monitored. The SuperPro Designer model follows the production protocols set out by Astellas, whose product yield is approximately 65% or 385kg of famotidine from 420kg TPN. The model examined various permutations of processing parameters which predicted yields of between 376.6kg and 395.5kg.

The presence of impurities makes modelling difficult as their weights had to be accounted for. The large quantity of data generated meant that not all permutations could be examined. SuperPro Designer is not able to be trained and iterations of each permutation are required to get meaningful data.

SuperPro Designer is used as a scheduling tool by many industries and allows for the same reactors to be used for different stages in the process. In Astellas however, each reactor has a single purpose which made modelling easier. Problems arose when a crossover between batch and continuous processes were merged. Sulphamide is recovered by Astellas and added to new sulphamide in each batch. However, this was not possible to model using SuperPro as the initial quantity of sulphamide (349.9kg) was being added to the recovered quantity (140kg) and each iteration increased the quantity of sulphamide in the reactor. The recovery of sulphamide was consequently omitted from the SuperPro Designer model.

SuperPro has been used in this instance to identify points in the process where losses occur. It has been somewhat successful in identifying the centrifuges as major points of loss. Once this has been achieved, further

modelling may be carried out using other software. For instance, computational fluid dynamics may be used to examine individual fluid flows in each of the unit operations and may provide more information than the overview provided by SuperPro.

5.2 Reasons for losses

Poor conversion rates from imidate to famotidine have been identified by the model as causing significant reductions in product formation. This is evident from the large amount of unreacted imidate present following the crystallisation of crude famotidine. Between 22% and 26% of imidate is predicted by the model to remain unreacted in VE-2300/2800. Further quantities of product are predicted to be lost as a result of the reactions in this reactor. The crystallisation step dictates that 99% of the pure famotidine present crystallises out of solution. Several problems have been encountered using crystallisation in the pharmaceutical industry and these are: (i) the control of supersaturation and particle size distribution, (ii) effective use of seed, (iii) efficient measurement of solubility's in multiple solvent systems to maximise purification and yield and (iv) the identification and retention of the most stable polymorphic form purification and yield (Kirwin and Orella, 2002). Precipitation of famotidine occurs when the cooled batch is seeded with pure famotidine crystals. Significant losses are incurred during centrifugation as dissolved famotidine is washed away in the centrifuges. Improved crystallisation will have a positive impact on the overall purity and yield of famotidine. Inadequate cooling periods for crystal generation will inhibit crystal formation and dissolved product will be washed into waste streams post centrifugation. However, energy balances were not considered for this thesis. Further analysis into the energy balances within the plant may highlight inadequacies in the process.

5.3 Concluding remarks

To verify the validity of the model, a sampling regime was organised with Astellas following a two week shutdown of the plant. It was envisioned that this period would allow residual pharmaceuticals to pass through the wastewater treatment facility. It was expected that a correlation between the quantity of analytes present and an increase in production would be observed. This did not occur. Instead, a peak in levels of both TPN and famotidine occurred in the fourth week of sampling. This is of significant importance to Astellas as it may equate to losses of 0.43% of product, or 1.635kg. Further analysis of the relevant process streams should be carried out in order to elucidate what is causing these losses. It is not only important from an economical viewpoint but as an environmental concern. Unaccounted losses of any chemicals in a pharmaceutical plant may have serious consequences to the renewal of environmental licences. Future work should entail mass flow analysis of the other pharmaceutical processes on-site along with water balances of all processes to narrow margins of error while modelling. This could be of high value to Astellas as it may highlight the locations of losses of not only products but also intermediates and raw materials. More precise analytical techniques are continually contributing to the tightening of regulations and pharmaceutical companies must pay careful attention to these laws.

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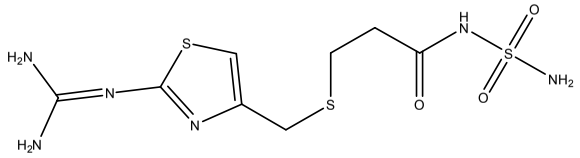
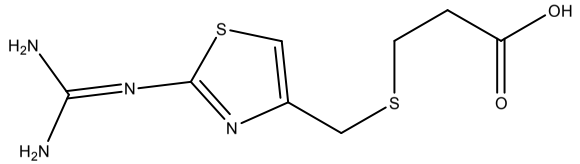
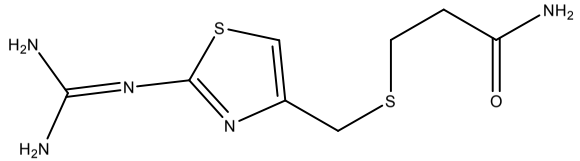
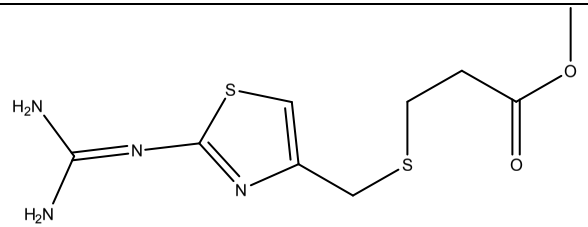
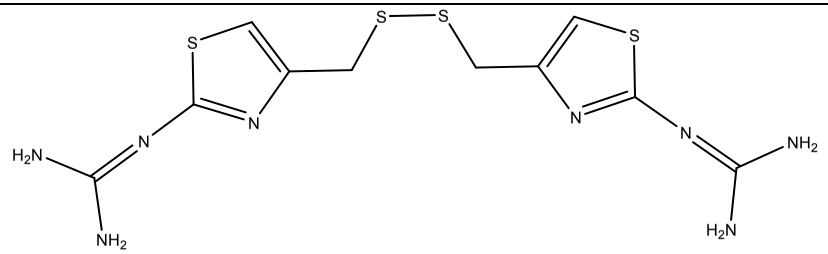
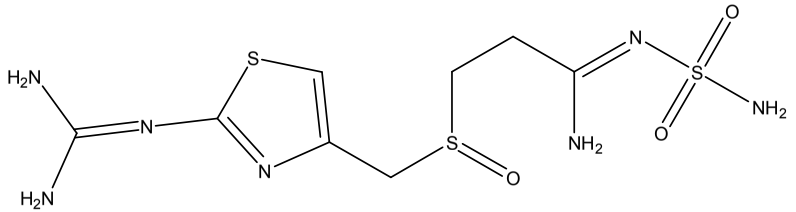
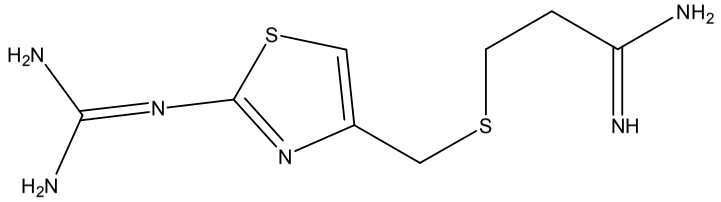
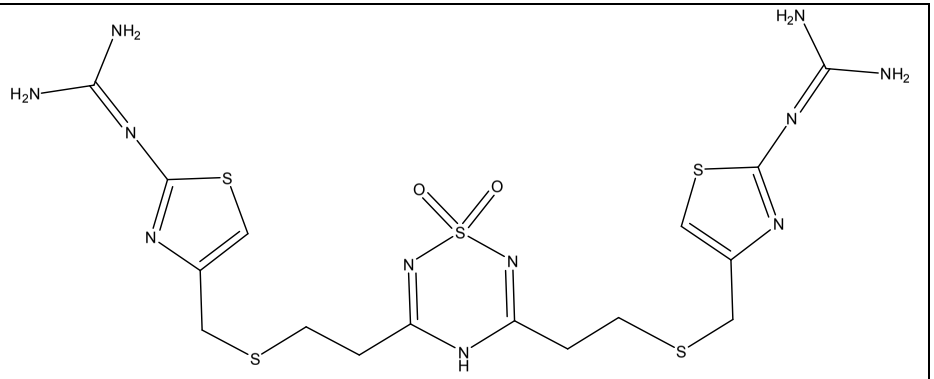
Appendices

Appendix A **Structures of the eight impurities observed in the production of famotidine.**

Impuritiy	IUPAC name	Molecular weight	Structure (Astellas)
A-1	3-[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio]-N-sulfamoylpropionamide	338	
A-2	3-[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio] propionic acid	260	
A-3	3-[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio] propionnamide.	259	
A-4	Methyl 3-[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio] propionate	274	

A-5	Bis[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl] disulphide	374	
A-6	3-[[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio] sulfinyl]-N-(sulfamoyl) propionamide	353	
A-7	3-[[[2-[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio] propionamide	258	

A-8	3,5-bis-[2-[[[(Diaminomethylene) amino]-4-thizolyl]-methyl]-thio]-ethyl]-4H-1,2,4,6-thiatriazine 1,1-dioxide	561	

Appendix B

Step 1 Assumptions which fit the average batch performance best

Assumption	**Value**
TPN purity	99%
TPN conversion to imidate	90%
A-3 formation from unreacted TPN	50%
A-2 formation from A-3	50%
A-4 formation from A-3	25%
Adherence to tank wall VE-2200	0.1%
Imidate retention in MA-2200	100%
TPN retention in MA-2200	80%
A-2 Retention in MA-2200	5%
A-3 Retention in MA-2200	95%
A-4 Retention in MA-2200	95%
A-5 Retention in MA-2200	95%

Step 2 Assumptions which fit the average batch performance best

Assumption	**Value**
Imidate conversion to CFM	76%
Crystallisation of CFM	99%
A-6 formation (A-3 + sulphamide)	10%
A-1 formation (A-2 + sulphamide)	80%
A-8 formation (famotidine + imidate)	0.1%
Imidate degradation to A-4 (water is rate limiting factor)	80%
A-1 retained in MA-2300/2800	10%
A-2 retained in MA-2300/2800	0%
A-3 retained in MA-2300/2800	10%
A-4 retained in MA-2300/2800	10%
A-5 retained in MA-2300/2800	10%
A-6 retained in MA-2300/2800	10%

A-7 retained in MA-2300/2800	10%
A-8 retained in MA-2300/2800	10%

Step 3 Assumptions which fit the average batch performance best

Assumption	**Value**
Adherence to tank wall in VE-2400	0.1%
Crystallisation of famotidine in VE2400	99%
Adherence to tank wall in VE-2500	0.1%
Adsorption of famotidine to carbon	0.5%

Step 4 Assumptions which fit the average batch performance best

Assumption	**Value**
Crystallisation of famotidine in VE-2600/4600	100%

Appendix C Range of preliminary investigated conversion rates of imidate to crude famotidine (10 – 100%) and the corresponding total dry weights.

Imidate Conversion (%)	A-1 (kg)	A-2 (kg)	A-3 (kg)	A-4 (kg)	A-6 (kg)	A-8 (kg)	C.FM (kg)	Dissolved CFM (kg)	Imidate (kg)	Sulphamide (kg)	TPN (kg)	Total (kg)
100	0.53	0.10	4.73	5.09	0.11	0	523.23	5.28	0.00	198.98	15.09	753.15
90	0.53	0.10	4.74	5.50	0.11	0.09	470.90	4.75	42.33	214.02	15.09	758.16
80	0.53	0.10	4.74	5.50	0.10	0.18	418.58	4.22	85.06	229.06	15.09	763.16
70	0.53	0.10	4.74	5.50	0.10	0.26	366.25	3.69	127.80	244.10	15.09	768.17
60	0.53	0.10	4.75	5.49	0.09	0.35	313.93	3.17	170.54	259.14	15.09	773.18
50	0.53	0.10	4.75	5.49	0.09	0.44	261.60	2.64	213.28	274.18	15.09	778.19
40	0.53	0.10	4.75	5.49	0.08	0.35	209.38	2.11	256.10	289.22	15.09	783.21
30	0.53	0.10	4.76	5.49	0.08	0.26	157.16	1.58	298.92	304.26	15.09	788.23
20	0.53	0.10	4.76	5.48	0.08	0.18	104.94	1.05	341.74	319.30	15.09	793.26
10	0.53	0.10	4.76	5.48	0.08	0.09	52.72	0.53	384.56	334.34	15.09	798.28

Appendix D Permutations of the parameters investigated up to VE-2300/2800. The green rows indicate those permutations which generated total crude famotidine in the range 405kg – 420kg and a purity of 96% or more. The values in the white rows were discarded.

Parameters modelled				Dry weights of process stream components									
TPN purity (%)	Imidate RXN Completion (%)	(%) Imidate retained MA-2200	Imidate conversion (%)	A-1 (kg)	A-3 (kg)	A-4 (kg)	A-5 (kg)	A-6 (kg)	A-8 (kg)	C.FM (kg)	TPN (kg)	TOTAL CFM (kg)	% Purity
100	91	99	78	0.52	4.69	5.44	0.00	0.10	0.19	408.11	0.15	419.22	97.351
100	91	99	76	0.52	4.69	5.44	0.00	0.10	0.21	397.65	0.15	408.77	97.280
100	90	100	76	0.58	5.22	6.04	0.00	0.10	0.21	397.25	0.17	409.57	96.991
100	90	99	78	0.58	5.22	6.04	0.00	0.10	0.19	403.63	0.17	415.94	97.042
100	90	99	76	0.58	5.22	6.04	0.00	0.10	0.21	393.28	0.17	405.60	96.963
99	92	99	76	0.46	4.12	4.80	3.94	0.10	0.21	398.00	0.13	411.76	96.657
99	92	99	74	0.46	4.12	4.80	3.94	0.10	0.23	387.52	0.13	401.30	96.566
99	91	100	76	0.52	4.65	5.39	3.94	0.10	0.21	397.65	0.15	412.60	96.376
99	91	100	74	0.52	4.65	5.39	3.94	0.10	0.23	387.18	0.15	402.15	96.277
99	91	99	78	0.52	4.65	5.39	3.94	0.10	0.19	404.04	0.15	418.97	96.435
99	91	99	76	0.52	4.65	5.39	3.94	0.10	0.21	393.68	0.15	408.63	96.341
99	90	100	78	0.58	5.17	5.98	3.94	0.10	0.19	403.63	0.17	419.76	96.158
99	90	100	76	0.58	5.17	5.98	3.94	0.10	0.21	393.28	0.17	409.43	96.057
98	93	99	76	0.40	3.56	4.16	7.88	0.10	0.21	398.26	0.12	414.69	96.038
98	93	99	74	0.40	3.56	4.16	7.88	0.10	0.23	387.78	0.12	404.23	95.931
98	92	100	76	0.46	4.08	4.75	7.88	0.10	0.21	397.96	0.13	415.57	95.762
98	92	100	74	0.46	4.08	4.75	7.88	0.10	0.23	387.48	0.13	405.11	95.649
98	92	99	76	0.46	4.08	4.75	7.88	0.10	0.21	393.98	0.13	411.59	95.722
98	92	99	74	0.46	4.08	4.75	7.88	0.10	0.22	383.61	0.13	401.24	95.608
98	91	100	76	0.51	4.60	5.34	7.88	0.10	0.21	393.64	0.15	412.42	95.445
98	91	100	74	0.51	4.60	5.34	7.88	0.10	0.22	383.28	0.15	402.08	95.324
97	95	99	74	0.28	2.50	2.96	11.83	0.10	0.23	392.07	0.08	410.05	95.616
97	94	99	76	0.34	3.01	3.54	11.83	0.10	0.21	398.43	0.10	417.56	95.420
97	94	99	74	0.34	3.01	3.54	11.83	0.10	0.23	387.95	0.10	407.09	95.298

Parameters modelled				Dry weights of process stream components									
TPN purity (%)	Imidate RXN Completion (%)	(%) Imidate retained MA-2200	Imidate conversstion (%)	A-1	A-3	A-4	A-5	A-6	A-8	C.FM	TPN	TOTAL CFM	% Purity
97	93	100	76	0.40	3.52	4.12	11.83	0.10	0.21	398.18	0.11	418.47	95.151
97	93	100	74	0.40	3.52	4.12	11.83	0.10	0.23	387.70	0.11	408.00	95.023
97	93	99	76	0.40	3.52	4.12	11.83	0.10	0.21	394.20	0.11	414.49	95.106
97	93	99	74	0.40	3.52	4.12	11.83	0.10	0.22	383.83	0.11	404.13	94.976
97	92	100	76	0.45	4.04	4.70	11.83	0.10	0.21	393.90	0.13	415.35	94.835
97	92	100	74	0.45	4.04	4.70	11.83	0.10	0.22	383.54	0.13	405.01	94.699

Appendix E Properties of the compounds used to produce famotidine.

Compound	**Molecular Wt.**	**Density g/cm^3**	**Melting Pt. (˚C)**	**Boiling Pt. (˚C)**
TPN	241.3	-	127-132	-
Sulphamide	96.11	-	89-93	-
Imidate	273.33	-	125-135	-
Famotidine	337.43	-	164	-
HCl gas	36.5	-	-	-
Dioxane	88.1	1.0329	11.	101
Methanol	32.04	0.7915	-97.8	64.7
Potassium Carbonate	138.2	-	-	-
Triethylamine	101.19	0.9445	114.7	89.3
N,N, Dimethylformamide	73.09	0.798	-61	153
Ethanol	46.07	1.07	-117.3	78.5
80% Acetic Acid	-	-	-8	-
NaOH pellet	40	-	-	-
Aq NaOH 25%	-	1.27	-17	-
Aq Sulphuric 35%	-	1.26	-86	-
Acetic Acid (glacial)	60.05	1.053	16.7	118
Dioxane/Methanol (2:1)	-	0.972	-7	-

Appendix F-i LC-MS responses of famotidine in the pH adjust tank.

Date	Sample A (259 m/z)	Sampe B (259 m/z)	Mean (259 m/z)	St. Dev	Conc. (mg/L)
05-Aug	13588	13646	13617	41.01	1.1
07-Aug	15284	15012	15148	192.33	1.23
10-Aug	14150	14248	14199	69.3	1.15
12-Aug	12226	11809	12018	294.86	0.97
14-Aug	69671	6782	38227	44469.2	3.1
19-Aug	16925	16279	16602	456.79	1.35
21-Aug	5455	6550	6003	774.28	0.49
26-Aug	11987	11629	11808	253.14	0.96
28-Aug	3373	3300	3337	51.62	0.27
01-Sep	51411	92771	72091	29245.9	5.85
02-Sep	44405	41829	43117	1821.51	3.5
03-Sep	69872	48252	59062	15287.7	4.79
10-Sep	58823	60217	59520	985.71	4.83
16-Sep	27889	27120	27505	543.77	2.23

Appendix F-ii LC-MS responses of famotidine in WWC1.

Date	Sample A (259 m/z)	Sample B (259 m/z)	Mean (259 m/z)	St. Dev	Conc. (mg/L)
05-Aug	33883	33882	33883	0.71	2.75
07-Aug	8927	10850	9889	1359.77	0.8
10-Aug	27584	25378	26481	1559.88	2.15
12-Aug	46053	43532	44793	1782.62	3.63
14-Aug	77257	69671	73464	5364.11	5.96
19-Aug	24946	23927	24437	720.54	1.98
21-Aug	9829	10484	10157	463.15	0.82
26-Aug	17264	12288	14776	3518.56	1.2
28-Aug	-	-	-	-	-
01-Sep	-	-	-	-	-
02-Sep	209749	197762	203756	8476.09	16.53
03-Sep	132564	143178	137871	7505.23	11.19
10-Sep	120999	127253	124126	4422.25	10.07
16-Sep	68038	62828	65433	3684.03	5.31

Appendix F-iii LC-MS responses of TPN in WWC1 after SPE. The concentration is calculated by the mean of the two samples the SPE concentration factor has been accounted for

	155	155	mean	stdev	Conc. (mg/L)
05-Aug	9212.00	8470.00	8841.00	524.67	0.041
07-Aug	10788.00	11024.00	10906.00	166.88	0.066
10-Aug	7568.00	8985.00	8276.50	1001.97	0.034
12-Aug	30411.00	28164.00	29287.50	1588.87	0.286
14-Aug	14462.00	14771.00	14616.50	218.50	0.110
19-Aug	44821.00	39679.00	42250.00	3635.94	0.441
21-Aug	15103.00	13927.00	14515.00	831.56	0.109
26-Aug	40918.00	-	40918.00	-	0.425
28-Aug	-	-	-	-	-
01-Sep	-	-	-	-	-
02-Sep	45550.00	29322.00	37436.00	11474.93	0.384
03-Sep	2411.00	2363.00	2387.00	33.94	nq
10-Sep	22475.00	8413.00	15444.00	9943.34	0.120
16-Sep	2823.00	21727.00	12275.00	13367.15	0.082

Appendix F-iv LC-MS responses of TPN in the pH adjust tank after SPE. The concentration is calculated by the mean of the two samples and the SPE concentration factor has been accounted for.

	155	155	mean	stdev	Conc. (mg/L)
05-Aug	3487.00	3962.00	3724.50	335.88	nq
07-Aug	-	-	-	-	peak tailing
10-Aug	6952.00	6207.00	6579.50	526.79	0.014
12-Aug	10208.00	10691.00	10449.50	341.53	0.060
14-Aug	5006.00	5169.00	5087.50	115.26	-0.004
19-Aug	9777.00	34650.00	22213.50	17587.87	0.201
21-Aug	5702.00	6253.00	5977.50	389.62	0.007
26-Aug	69636.00	102464.00	86050.00	23212.90	0.967
28-Aug	82593.00	70765.00	76679.00	8363.66	0.854
01-Sep	8155.00	7900.00	8027.50	180.31	0.031
02-Sep	9423.00	9939.00	9681.00	364.87	0.051
03-Sep	-	-	-	-	-
10-Sep	66876.00	47313.00	57094.50	13833.13	0.619
16-Sep	644.00	2823.00	1733.50	1540.79	-0.044

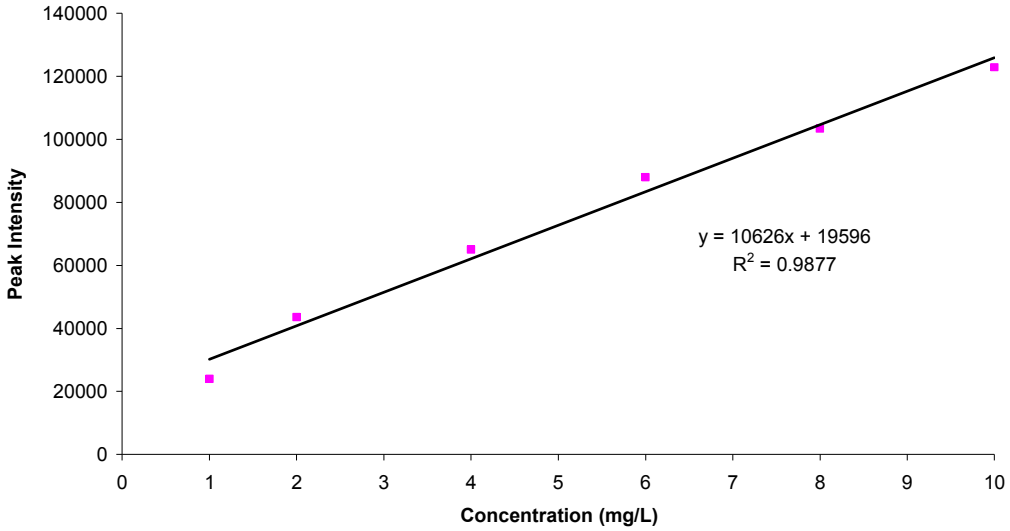
Appendix G-i Comparison of the solvent composition in Astellas at the pH adjust tank and those in an example of the model (Ettarh, 2008).

pH Adjust tank (Ettarh, 2008)		**SuperPro pH adjust tank**	
Component	**% Composition**	**Component**	**% Composition**
Dimethylformamide	5.35	A-1	0.0028
1,4 Dioxane	1.39	A-2	0.0524
Ethanol	11.75	A-3	0.0268
Ethylacetate	0.06	A-4	0.031
Inorganic Residue	1.06	A-5	0.0256
Methanol	1.09	A-6	0.0006
Toluene	0.02	A-8	0.0013
Triethylamine	0.01	Acetic-Acid	0.0005
Water	81.71	Ammonia	0.0054
Total	102.44	B-Form Famotidine Dissolved	0.0245
		Carbon Dioxide	0.3995
		Activated Carbon	0.1846
		1,4 Dioxane	0.0595
		Dissolved SPFM	0.0387
		Dimethylformamide	0.0816
		Ethanol	0.1448
		Imidate	0.0293
		KCl	2.0805
		Methanol	0.0223
		PFM	0.012
		K_2CO_3	6.7034
		Sodium Acetate	0.7451
		Sodium Hydroxide	0.2611
		TPN	0.0112
		Triethylamine	0.002
		Water	89.0535
		Total	100.0

Appendix G-ii Comparison of the solvent composition in Astellas at WWC1 and those in an example of the model (Ettarh, 2008).

Thermal Treatment (Ettarh, 2008)		**SuperPro Thermal Treatment**	
Component	**% Composition**	**Component**	**% Composition**
Dimethylformamide	1.14	A-1	0.0001
Ethanol	30.32	A-2	0.0013
Ethylacetate	14.07	A-3	0.0001
I,4 Dioxane	4.37	A-4	0.0001
inorganic Residue	0.09	A-5	0.0001
Methanol	31.27	A-6	0.0
Toluene	11.37	A-8	0.0
Triethylamine	1.28	Ammonia	0.0004
Water	4.47	1,4 Dioxane	13.4294
Total	98.38	Dissolved CFM	0.0593
		Dimethylformamide	20.2618
		Ethanol	33.6383
		Imidate	1.4988
		Methanol	25.4887
		Sulphamide	1.4498
		TPN	0.2281
		Triethylamine	1.7872
		Water	2.1564
		Total	100.0

Appendix H-i Standard curve of famotidine (0 – 10mg/L) using LC-MS.



Appendix H-ii Standard curve of TPN (0 – 10mg/L) using LC-MS